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Screening Submersed Plant Species for Phytoremediation of Explosives-Contaminated Groundwater from the Milan Army Ammunition Plant, Milan, Tennessee

by Elly P. H. Best, AScI Corporation

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Prepared for U.S. Army Environmental Center

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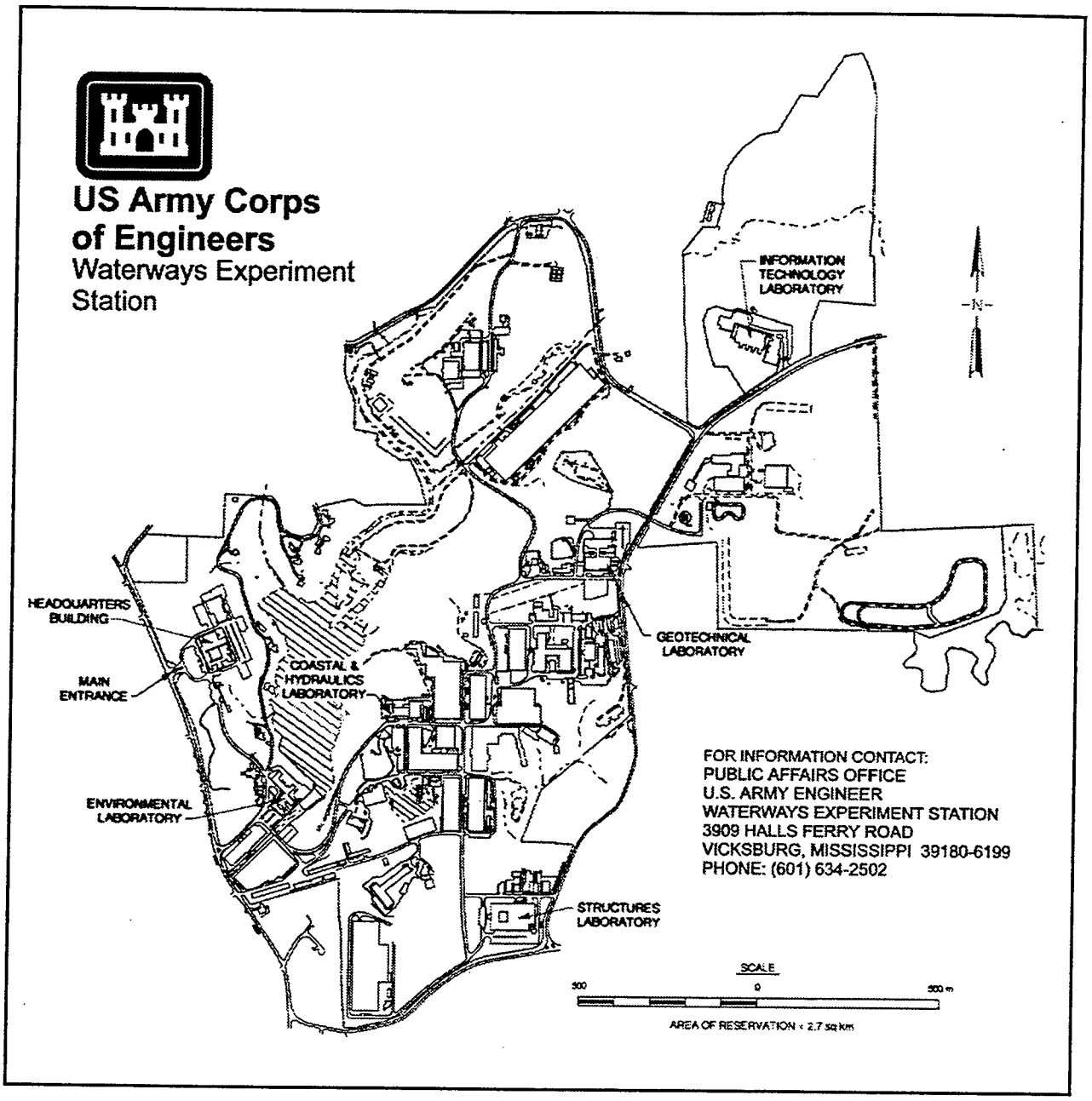

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Preface

The work reported herein was conducted as part of the Army Environmental Center's "Phytoremediation of Explosives-Contaminated Groundwater Using Constructed Wetlands" project under a partnering agreement involving the U.S. Army Environmental Center, as the lead agency with Ms. Darlene F. Bader, SFIM-AEC-ETD, as project manager, and the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, and the Tennessee Valley Authority, Muscle Shoals, AL, providing technical support. Funding was provided under the DoD's Environmental Security Technology Certification Program.

This investigation was conducted at WES under the general supervision of Dr. John Harrison, Director, Environmental Laboratory (EL), and Dr. Richard E. Price, Chief, Environmental Processes and Effects Division (EPED), EL.

The work reported herein was carried out with the invaluable technical help of Ms. Anne B. Stewart, AScI Corporation, WES, and Mr. Robbie B. Godwin, Ecosystem Processes and Effects Branch (EPEB), EPED. Analysis of explosives and TNT degradation products in water was performed by Mr. Mike Ochman and Ms. Margaret Richmond, AScI Corporation, WES. Analysis of explosives and degradation products in plants was performed by the Environmental Chemistry Branch, Environmental Engineering Division, EL. Nutrients, metals, and ions in water were determined by Mr. David Honnell, AScI Corporation, Lewisville Aquatic Ecosystem Research Facility. Various components in the sediments were determined by Ms. Susan Fox of AScI Corporation, WES.

The financial support of the Strategic Environmental Research and Development Program is gratefully acknowledged.

The report was prepared by Drs. Elly P. H. Best, AScI Corporation, and Susan L. Sprecher, Herbert L. Fredrickson, and Steven L. Larson, EL. Technical reviews of this report were provided by Drs. Judy Pennington and Douglas Gunnison, EL. Statistical counseling was provided by Ms. Joan Clarke, Fate and Effects Branch, EPED. Valuable discussions and comments during the course of the study were graciously provided by Drs. Pennington and William Davis, EPEB.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Robin R. Cababa, EN.

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1 Introduction

Phytoremediation of Explosives

Concerns about the environmental fate of explosive residues and transformation products present in soil and groundwater at military installations and ammunition plants have compelled a Department of Defense (DoD) focus on cost-effective remediation technologies (Walsh 1990). Most current techniques involve pumping water to the surface where it can be treated physically (adsorption to granular activated carbon columns; ultraviolet radiation) or chemically (oxidation via hydrogen peroxide and ozone) to remove explosives (Zappi 1995). Biological degradation presents another option for treatment in these situations. While bioremediation research has targeted the role of soil microorganisms in explosives degradation (Carpenter et al. 1978; Kaplan and Kaplan 1983; McCormick, Cornell, and Kaplan 1981, 1984; Sublette, Ganapathy, and Schwartz 1992; Major, Bollag, and Amos 1994; Pennington et al. 1995), information on plant enzyme-mediated processes is increasing (Mueller et al. 1995; Van Beelen and Burris 1995). Plant-enhanced degradation, or phytoremediation, of 2,4,6-trinitrotoluene (TNT)[1] by terrestrial and aquatic macrophytes has been proposed as a promising groundwater treatment process (Schnoor et al. 1995).

The Milan Army Ammunition Phytoremediation Demonstration Project

Under a partnering agreement between the U.S. Army Environmental Center, the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, and the Tennessee Valley Authority (TVA), Muscle Shoals, AL, the demonstration project "Phytoremediation of Explosives-Contaminated Groundwater Using Constructed Wetlands" was funded by the DoD Environmental Security Technology Certification Program (ESTCP). The U.S. Army Environmental Center, as lead agency, selected Milan Army Ammunition Plant (MAAP), near Milan, TN (longitude 88° 50' W, latitude 35° 45' N), as the demonstration site on

[1] For convenience, symbols and unusual abbreviations are listed and defined in Appendix D.

the basis of the high concentrations of TNT and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX, "royal demolition explosive") in groundwater at the site, significantly above potable water levels of 2 $\mu g\ L^{-1}$ (2 ppb) for each compound mandated by the U.S. Environmental Protection Agency (USEPA 1989).

Phase I of this project provided for laboratory-scale plant screenings by WES and TVA to evaluate locally adapted aquatic and wetland species for their differential ability to diminish levels of TNT and RDX and byproducts in MAAP groundwater. These findings, reported as biomass-normalized kinetic constants k for TNT and RDX removal, then supported species selection for the Phase II field-scale constructed wetland demonstration deployed by TVA at MAAP. WES evaluated submerged aquatic species for use in lagoons, and TVA tested emergents for culture in subsurface-flow gravel beds, under common conditions formulated in a standard protocol. The design calculations of both the lagoons and the gravel wetlands were based on a total hydraulic retention time of 10 days and a minimum flow rate of 19 L min^{-1} (5 gal min^{-1}) to each system (Behrends et al. 1996).

This report presents the results of the WES study to quantify the ability of 10 species of submerged aquatic plants, adapted to lentic habitats in western Tennessee, to phytoremediate explosives-contaminated groundwater. Species evaluated under hydroponic batch conditions were *Myriophyllum spicatum* L. (Eurasian watermilfoil), *Egeria densa* Planch. (egeria, Brazilian elodea), *Elodea canadensis* Rich. in Michx. (elodea, waterweed), *Vallisneria americana* Michx. (vallisneria, wildcelery, tapegrass), *Potamogeton crispus* L. (curlyleaf pondweed), *Potamogeton pectinatus* L. (sago pondweed), *Heteranthera dubia* (Jacq.) MacM. (water star-grass), *Eleocharis parvula* (R. & S.) Link (dwarf spikerush), and *Chara vulgaris* L. (stonewort, muskgrass). The emergent parrot-feather (*Myriophyllum aquaticum* (Vell.) Verdc.) was included in both the TVA and WES screening evaluations to provide a comparison between the screening tests since this species has been shown to degrade TNT (Schnoor et al. 1995). The Phase I screenings also assessed the effects of plant density and fertilization on the extent and rate of contaminant removal by these species in native and heat-inactivated sediments.

2 Materials and Methods

This study was carried out under the general conditions described in a protocol for standardized phytoremediation screening formulated jointly for this project by the WES and the TVA in September 1995 (Appendix A).

Plant Material

Nine submerged species were selected by WES for this survey based on a review of desirable growth habit features in species adapted to the Milan area, information on their remediation-related metabolism, and availability of material for testing and eventual deployment in the treatability and Phase II studies. A tenth species, parrot-feather, was included for purposes of comparison to related studies.

Species selection

The array of native and introduced submerged species adapted to lentic habitats in western Tennessee was reviewed (Godfrey and Wooten 1979, 1981; Westerdahl and Getsinger 1988; Tennessee Valley Authority, no date) and evaluated for growth habit and ecological characteristics deemed to be of value in aquatic plant lagoons for groundwater phytoremediation. These traits included perenniality, to provide year-round presence of biomass and increased density; production of high biomass and leaf surface area within the water column; extensive root and rhizome systems, to support natural propagation, substrate stabilization, and remediation-enhancing interactions with microbial flora in sediment; and the water depth to which the plant was adapted. Attention was given to whether species were exotic or native, and whether they were considered as noxious weeds in Tennessee. However, weedy species were not automatically eliminated.

Where available, data on nitroreductase activity were taken into account. This enzyme has been shown to initiate TNT degradation by chemical reduction of NO_2 groups to $-NH_2$, and is suggested to be present in many plants, including the alga *Chara*, and the wetland/aquatic angiosperms *Eleocharis* spp., *Potamogeton*

pusillus L., *Hydrilla verticillata* (L. f.) Royle (hydrilla), as well as parrot-feather (Schnoor et al. 1995; data from L. H. Carreira, in Best et al.[1] 1996, 1997).

A site visit to MAAP on 13 September 1995 (Appendix B) allowed project participants to become familiar with local aquatic and wetland communities and review submersed and emergent candidates for laboratory evaluations, as well as for the Phase II field demonstration. Completely submerged species were rare in the water bodies examined within 24 km (15 miles) of the MAAP; only *Cabomba caroliniana* Gray (fanwort) was found. However, parrot-feather was abundant in the area.

Finally, availability of planting material in mid-September was determined. The species chosen are listed in Table 1.

Source and acclimation of planting material

Parrot-feather was obtained through TVA from a population growing in a pond near Muscle Shoals, AL; plants from the same source were included in the TVA emergent screening. All other species, except stonewort, were provided from populations cultured in outdoor ponds at the Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX. Stonewort was obtained from a commercial nursery (Southern Tier Consulting, West Clarkville, NY).

Plants, except for stonewort, were acquired during the last two weeks of September 1995 and held in hydroponic monocultures in a WES greenhouse, using a 0.25x Hoagland's nutrient culture medium (Hoagland and Arnon 1938). Stonewort had previously been planted into sediment in a low-alkalinity solution (Smart and Barko 1985). Cultures were aerated to enhance mixing and air/water CO_2 exchange.

Most of the plant material was received as unrooted apical shoots; vallisneria and spikerush were received as whole plants comprising rooted crowns. Rooting of apical shoots during the acclimation period was minimal.

Groundwater

Explosives-contaminated groundwater used for screening by both WES and TVA originated from a single batch collected from MAAP Well MI 146 and transported in a tank truck to TVA. A subsample of 833 L (220 gallons) was brought to WES in four stainless steel 208-L (55-gallon) drums at the end of September and stored at room temperature before use. Initial nutrient and explosives

[1] E. P. H. Best, M. E. Zappi, H. L. Fredrickson, S. L. Sprecher, and J. Miller (1996). "Optimization of constructed phytoremediation systems for treatment of contaminated groundwater at the Iowa Ammunition Plant. Phase I: Site reconnaissance; Phase II: Batch testing," Letter Report for the U.S. Army Engineer District, Omaha, Omaha, NE.

Table 1
Submersed Aquatic Plant Species Used in Factorial Screening for Explosives Removal, WES, October 1995

Group	Family	Plant Species	
		Scientific Name	Common Name
Angiosperms			
Monocotyledons	Hydrocharitaceae	*Egeria densa* Planch.	Egeria
		Elodea canadensis Rich. in Michx.	Elodea
		Vallisneria americana Michx.	Vallisneria
	Potamogetonaceae	*Potamogeton crispus* L.	Curlyleaf pondweed
		Potamogeton pectinatus L.	Sago pondweed
	Pontederiaceae	*Heteranthera dubia* (Jacq.) MacM.	Water star-grass
	Cyperaceae	*Eleocharis parvula* (R.&S.) Link	Dwarf spikerush
Dicotyledons	Haloragaceae	*Myriophyllum aquaticum* (Vell.) Verdc[1]	Parrot-feather
		Myriophyllum spicatum L.	Eurasian watermilfoil
Algae			
Algae	Characeae	*Chara vulgaris* L.	Stonewort

[1] Comparison species.

composition of this test water was characterized at WES in three 100-ml samples of a blend of equal volumes taken from each drum (Table 2). Thus, the plants screened at WES were subjected to levels of approximately 2.2 mg L^{-1} (ppm) TNT and 3.0 mg L^{-1} RDX.

Sediment

Sediment used as a component of experimental controls originated from dry-land soil collected in a low-lying grassland area, which had not been fertilized for the last 5 years, near the X production line at MAAP. Soil was excavated at the end of September 1995, placed in polypropylene 19-L (5-gallon) buckets, transported to WES, and stored in a cold room (5 °C). The soil was prepared for the experiment by wetting with tap water and fully blending the contents of one bucket in a mechanical mixer. Dry weight was determined from a 34-g wet weight sample. A portion of this sediment was autoclaved (1 hr at 120 °C and 1.03 atmospheres (15psi)), mixed, and autoclaved again (30 min at 120 °C and 1.03 atmospheres (15 psi)) to inactivate soil organisms and enzymes. Both autoclaved and unautoclaved sediment controls were used.

Table 2
Chemical Characteristics of the MAAP Groundwater from Initial Characterization at WES

Parameter	Value
pH	8.3 ± 0.1
Macro-, Micronutrients, mg L^{-1}	
Alkalinity	15 ± 3
NO_3-N	5.8 ± 1.7
NH_4-N	0.08 ± 0.08
SRP	0.179 ± 0.034
SO_4	1.53 ± 0.16
Ca	5.9 ± 1.3
Explosives, µg L^{-1}	
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	NA[1]
2,6-Diamino-,4-nitro-toluene (2,6DANT)	73.9 ± 2.6
2,4-Diamino-,6-nitrotoluene (2,4DANT)	6.6 ± 1.5
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	3002.2 ± 82.0
1,3,5-Trinitro-benzene (TNB)	308.2 ± 16.6
1,4-Dinitro-benzene (1,4DNB)	–[2]
1,3-Dinitro-benzene (1,3DNB)	29.2 ± 14.2
Nitrobenzene (NB)	–[2]
2, 4, 6-Trinitrotoluene (TNT)	2196.7 ± 68.1
2-Amino-dinitrotoluene (2ADNT)	43.2 ± 0.6
4-Amino-, 2, 6-dinitrotoluene (4ADNT)	35.7 ± 0.9
2,4-Dinitrotoluene (2,4DNT)	–[2]
2,6-Dinitrotoluene (2,6DNT)	–[2]
2-Nitrotoluene (2NT)	–[2]
4-Nitrotoluene (4NT)	–[2]
3-Nitrotoluene (3NT)	–[2]

Note: Mean values and standard deviations of samples taken from three barrels.
[1] Not analyzed.
[2] <0.1 µg L^{-1}.

Experimental Design

The experiment was set up as a randomized complete block design, in which each of the three blocks contained one experimental unit of every treatment level evaluated: 10 plant species, each at two densities and at one fertilizer amendment of the lower density; a groundwater control without plants; and unautoclaved and autoclaved sediment controls with groundwater but without plants. This allowed statistical testing of treatment effects without requiring an inordinate number of experimental units. However, stonewort was not tested at the higher density due

to a lack of planting material, and thus each block contained 32 experimental units, for a total of 96.

Experimental Conditions

The screening was carried out over a 10-day incubation period, 3-13 October 1995, in a large walk-in controlled environment growth chamber. Experimental units were glass aquaria, 15 by 15 by 37.5 cm high, constructed with silicone sealant. After plants or sediment was placed in them, aquaria were filled with groundwater to a final depth of 15 cm, giving a uniform total test volume (rather than total volume of liquid) of 3.375 L. Plants were incubated without mechanical support as approximately 15-cm apical shoots or as whole crowns at 9 g fresh weight (FW) L^{-1} (D1: representative of biomass levels for a single aquatic species at the height of the growing season; Appendix A), and 18 g FW L^{-1} (D2), giving 30.4 or 60.8 g plant material per aquarium. As an emergent aquatic, parrot-feather was expected to have approximately half its biomass above the water surface; therefore, twice as much material was incubated (60.8 g and 121.6 g). A weighed portion of sediment (255 to 270 g) was placed in aluminum foil-lined, stainless steel trays in aquaria. Filled aquaria were covered with glass lids (except in the case of parrot-feather) to minimize evapotranspiration.

To test the effect of nitrogen (N) fertilization on explosives removal, groundwater was amended with 50 mg NO_3-N L^{-1} (F2). This was applied as 1.22 g KNO_3 to the designated aquaria and dissolved in water before plants were added. Unamended units were designated as F1.

High-pressure sodium and metal halide lamps provided a photosynthetic spectrum at a level of 400 to 500 $\mu E\ m^{-2}\ s^{-1}$ at 22.5 cm above the water surface. Each block of experimental units was positioned within an area of similar light intensity. Sides of aquaria were covered with black curtains to exclude incident light. An automatic timer provided a day length of 12 hr, and temperature was set at 25 °C.

Culture solutions were not aerated to (a) mimic expected slow water flow under field conditions and (b) produce the low oxygen (O_2) concentrations under which RDX removal was shown to be enhanced in a recent TVA study[1].

Experimental Procedures and Sampling

At the beginning of the test, groundwater was pumped from individual barrels into each aquarium, and predetermined weights of fresh plant material, sediment, or nitrogen were added to initiate incubation. Water samples were taken 1, 4, 12,

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[1] F. J. Sikora, personal communication, September 1995, Tennessee Valley Authority (TVA), Muscle Shoals, AL.

24, and 240 hr (10 days) after incubation began. Prior to sampling, the contents of the aquarium were mixed using a glass rod. The 100-ml water sample was collected into a glass cylinder and decanted into a glass bottle with a Teflon-lined cap. Water samples were refrigerated (5 °C) in the dark until further processing, usually within 24 hr of collection.

After the final water sampling (240 hr), plant materials were removed and weighed. A dry weight:fresh weight (DW:FW) ratio was determined for each species by drying a weighed portion of material in a ventilated oven at 70 °C until constant weight was attained and reweighing. Relative growth rates were calculated by dividing the natural log (ln) transform of final plant DW by initial DW, and dividing by the 10 days of incubation. Sediment was removed, weighed, placed in glass jars, and kept refrigerated until analysis. A 1-L sample of water was placed into a plastic bottle, pH was measured, and the bottle was placed in a freezer (-20 °C) to await nutrient analysis. Oxygen concentration was measured within the aquarium using a YSI O_2 electrode.

Chemical Analysis

Analytical specifications, calibration compounds, and method references are described in Appendix C.

Explosives in water

Levels of explosives and their metabolic/degradation products in all water samples were determined at WES. These 100-ml water samples were concentrated using a solid-phase extraction (SPE) method; explosives were eluted in acetonitrile and analyzed using high-performance liquid chromatography (HPLC), using a method based on EPA Method 8330 (U.S. Environmental Protection Agency 1992; Jenkins et al. 1995) (see Appendix C). Detection limits for explosives with these methods were 0.1 $\mu g\ L^{-1}$. Azoxy compounds were measured only in the 10-day water samples of one block, due to the lengthy procedure required for their analysis.

Cartridge-SPE was used here to provide an approximately 100x preconcentration of water samples, since it was anticipated that explosives concentration would be low (< 2 $\mu g\ L^{-1}$) in many treatments following 10-day static incubations. This step was expected to allow detection of levels of ≥ 0.1 $\mu g\ L^{-1}$ TNT and RDX in groundwater samples.

However, it was found that water in which plants had been incubated contained organic matter particulates that adsorbed to the polymer used in the SPE (Waters RDX cartridges, No. 47220), sometimes increasing the throughput time of samples and interfering with desorption of explosives during the elution phase. This probably contributed to variance in replicate means, particularly for RDX.

A comparison was made of compounds and concentrations found by separate WES and TVA analyses of initial samples of the common batch of MAAP groundwater used for screening. The results are listed in Table C1.

Explosives in plant material and sediment

Levels of TNT, RDX, and certain metabolic/degradation products of TNT were determined in plants from the lower density incubations and in unfertilized sediment controls. Plant samples were quick-frozen in liquid N_2, then ground to a fine powder. Two-gram FW portions were extracted in 10 ml acetonitrile by an 18-hr sonication in a water-cooled (5 °C) sonic bath. Samples were then centrifuged at 2450 x g for 5 min. Five milliliters of the extract supernatant was placed on a clean-up column consisting of 0.5 g Florisil overlaid with 0.5 g neutral alumina. The column was washed with 5 mL of acetonitrile, and the resulting extract was diluted 1:1 with deionized water and analyzed by HPLC (EPA Method 8330). Two-gram portions of sediment were analyzed using the same method but without grinding.

Alkalinity, macronutrients, and calcium in water

Alkalinity, pH, nitrogen (NH_4-N, NO_3-N), available phosphate phosphorus (P-PO_4), soluble reactive phosphorus (SRP), sulphate (SO_4), and total calcium (Ca) were analyzed at Lewisville Aquatic Ecosystem Research Facility, using methods outlined in Appendix C.

Macronutrients, bulk density, and organic matter in sediment

These determinations were made at WES (Table 3). Total Kjeldahl N and phosphorus (P) were determined in soil digests, and measured colorimetrically. Exchangeable ammonium and SRP were determined with standard methods (Appendix C).

Table 3
Mean Values and Standard Deviations (N=3) of Wetted Milan Soil Used in the Experiment

Parameter	Unit	Concentration
Nitrogen	g.kg DW^{-1}	1.4659 ± 0.055
Exchangeable NH_4-N	g.kg DW^{-1}	0.007 ± 0.000
Phosphorus	g.kg DW^{-1}	0.447 ± 0.014
Available PO_4-P	g.kg DW^{-1}	0.067 ± 0.002
Bulk density	g $DW.ml^{-1}$	1.246 ± 0.009
Moisture	g H_2O.kg FW^{-1}	26.91 ± 0.78
Organic matter	g.kg DW^{-1}	3.96 ± 0.13

Data Analysis

Final summary and analysis of explosives concentrations were carried out on TNT and RDX separately, using Statgraphics Plus (Version 7; Statistical Graphics Corporation, Bitstream Inc., Cambridge, MA) to perform analysis of variance (ANOVA), regression analysis, multiple range tests (Tukey's honest significant difference (HSD)), principal component analysis (PCA), and correlation analysis (Spearman Rank correlation). Significance was tested at the 95 percent confidence level, $P \leq 0.05$.

Since most of the groundwater used for incubations came from one barrel, the initial explosives concentration of that barrel was used for calculations at time zero: 2,123 $\mu g\ L^{-1}$ for TNT and 2,934 $\mu g\ L^{-1}$ for RDX. Where log transformation of data on nitrobody concentrations that were below detection was required, the detection level was used in place of zero. For TNT and RDX, this was 0.1 $\mu g\ L^{-1}$.

Data from HPLC analyses of all water samples were initially screened for outliers using a method based on Hotelling's T-square (Hotelling 1953); however, due to high between-treatment variability, this excluded whole species or factors and was not informative. Subsequently, only those samples thought to have been incorrectly prepared for analysis or misinjected during HPLC (an absence of peaks following the injection peaks in the chromatograms) were excluded. These amounted to 9 samples out of a total of 540, or 1.7 percent.

Initial ANOVA of TNT and RDX data as randomized complete block experiments showed that interblock differences were not statistically significant ($P = 0.576$ and 0.207 for TNT and RDX, respectively). Data sets were subsequently analyzed as completely randomized designs with three replications, without subtracting block effects. ANOVA and HSD comparisons among species and factors were carried out on data sets including all six sampling times, thereby identifying treatments with lower concentrations throughout the incubation period.

3 Results and Discussion

TNT Concentration in Water: Effects of Species, Density, and Fertilization

TNT removal

During the 10-day static exposure, removal of TNT from groundwater was more rapid from incubations with plants than from water or sediment controls lacking plants, and treatments within each amendment factor behaved similarly (Figure 1, Table 4). Treatment effects were evident by 4 hr, when there were significant differences in TNT levels between plant treatments and controls under all factors tested (Table 4, $P < 0.001$, data not shown). However, by 10 days all treatments had effected removal of 97 percent or more of the original 2,123 µg TNT L^{-1}, except for unamended groundwater and sediment controls, which remained at 471 and 450 µg TNT L^{-1}, respectively, and fertilized groundwater controls (at 685 µg L^{-1}). Among plant incubations, only egeria and parrot-feather retained TNT concentrations above detection limits at the end-point of the study (Table 4).

Doubling plant biomass to 18 g FW L^{-1} (D2) accelerated the decrease in TNT concentration (Table 4). TNT concentrations in water incubated with sago pondweed at this density had decreased below detection limits by 12 hr, while it remained at 1,775 µg L^{-1} in groundwater lacking plants. Nitrogen amendment of incubation water (F2) produced little change in TNT removal with plants at the lower density, and fertilization of the groundwater control was associated with the least TNT removal of any treatment (Table 4).

ANOVA of all sampling time data points by treatment within factors of density and fertilization showed that both treatment and time significantly affected TNT concentration ($P < 0.001$; data not shown). The associated multiple-range tests (Table 5) showed that seven species--elodea, sago and curlyleaf pondweeds, stonewort, Eurasian watermilfoil, water star-grass, and parrot-feather--differed significantly from groundwater controls in removal over time under all three treatment conditions. With higher plant density, spikerush and vallisneria treatments also outperformed the groundwater control, while elodea, sago pondweed, and water star-grass differed from all three controls. Elodea enhanced removal over that of

Table 4
Mean Values (N = 3) of TNT Concentrations, $\mu g\ L^{-1}$, in Groundwater over 10-Day Incubation with Plant Species at Two Densities and Controls, and with Plant Species at Lower Density and N Fertilization

Treatment	Incubation Period, hr						Removal[1], %
	0	1	4	12	24	240	
Lower Density - 9 g FW L^{-1}							
Parrot-feather	2123	1431	887	375	37	16	99
Milfoil	2123	1354	473	173	24	–[2]	100
Egeria	2123	1476	1183	843	407	34	98
Elodea	2123	1181	381	102	23	–[2]	100
Vallisneria	2123	1592	1084	902	226	–[2]	100
Curlyleaf p'weed	2123	1255	613	406	51	–[2]	100
Sago pondweed	2123	1209	462	245	24	–[2]	100
Star-grass	2123	1344	679	414	56	–[2]	100
Spikerush	2123	1467	775	560	102	–[2]	100
Stonewort	2123	1506	315	216	65	–[2]	100
Higher Density - 18 g FW L^{-1}							
Parrot-feather	2123	1077	436	32	4	–[2]	100
Milfoil	2123	1139	215	26	13	–[2]	100
Egeria	2123	1380	877	253	93	4	100
Elodea	2123	565	108	6	–[2]	–[2]	100
Vallisneria	2123	1366	788	169	48	–[2]	100
Curlyleaf p'weed	2123	1027	385	19	10	–[2]	100
Sago pondweed	2123	830	246	–[2]	–[2]	–[2]	100
Star-grass	2123	879	261	31	–[2]	–[2]	100
Spikerush	2123	1360	694	157	9	–[2]	100
Controls							
Groundwater	2123	1841	1797	1775	1068	471	78
Sediment	2123	1453	1736	1439	1018	450	79
Autocl.sediment	2123	1661	1700	872	1039	25	99
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1}							
Parrot-feather	2123	1333	947	151	32	–[2]	100
Milfoil	2123	1081	609	87	31	–[2]	100
Egeria	2123	1533	1000	406	221	39	98
Elodea	2123	1094	490	77	17	–[2]	100
Vallisneria	2123	1446	1105	430	230	–[2]	100
Curlyleaf p'weed	2123	1349	806	270	72	–[2]	100

(Continued)

[1] Based on concentrations at 10 days.
[2] Less than detection limit of 0.1 $\mu g\ L^{-1}$.

Table 4 (Concluded)

Treatment	Incubation Period, hr						Removal[1], %
	0	1	4	12	24	240	
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1} (Continued)							
Sago pondweed	2123	1207	536	94	41	-[2]	100
Star-grass	2123	1421	652	241	59	-[2]	100
Spikerush	2123	1246	730	296	61	-[2]	100
Stonewort	2123	1403	489	118	51	-[2]	100
Fertilized Controls							
Groundwater	2123	1922	1728	1208	1496	685	68
Sediment	2123	1900	1758	1343	1263	57	97
Autocl.sediment	2123	1631	1439	1106	909	-[2]	100

unautoclaved sediment under N-amendment. However, no significant differences in activity among species were shown by this test.

Comparison of the effects of amendment factors on activity within each individual species showed that neither increase in biomass nor fertilization significantly enhanced removal ($P>0.560$, data not shown).

Removal kinetics and correlated effects

In order to examine kinetic differences among treatments, the exponential regression model $Y = exp\ (a + bX)$, where Y = concentration, X = time, and slope is negative, was used to describe the exponential decrease in TNT seen under the three conditions tested (Table 6). These regression statistics were used to extrapolate hydraulic retention time in days required to reach a cleanup level of 2 µg TNT L^{-1}, where $t_{2\mu g} = [(ln\ 2 - a)/b]/24$. The removal rate constant, K, was taken as the slope of the regression and normalized for DW biomass. This metric is independent of intercept.

Regression parameters of the fitted curves were examined for differences among treatments and factors (Table 6). In general, R^2 values were highest and most consistent for plants at the lower density, indicating smaller variances among data from this factor (see also Figure 1). It is possible that nutrient limitation and low O_2 concentrations in water, resulting from the greater amount of plant material incubated, contributed to variability at the higher density. The average intercept and slope of plant treatments across all three factors, D1, D2, and F1, - were lower and more negative than those of controls (for both, $P<0.001$, data not shown). However, plant treatment intercepts did vary among factors, ranging similarly for D1 and F2, and dropping significantly in D2 ($P < 0.001$, data not shown). This suggests that higher plant density was associated with decreased TNT concentration in the early part of the incubation and may be related to adsorption.

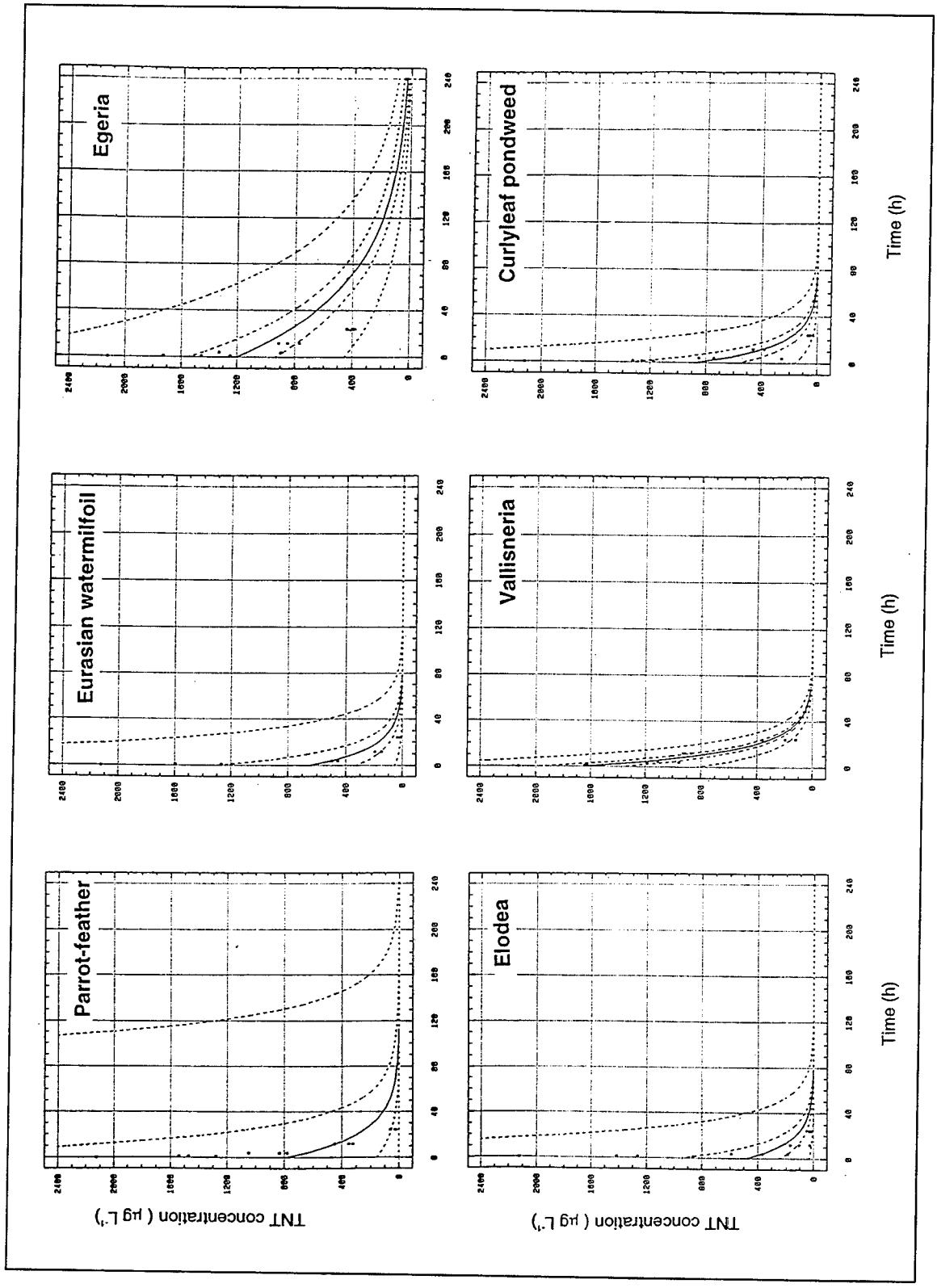

Figure 1. Changes in TNT concentration over time of groundwater incubated with one of ten aquatic plant species, alone or with unautoclaved or autoclaved sediment. Solid lines, fitted curves; interrupted lines, 95 and 90 percent confidence levels (Sheet 1 of 3)

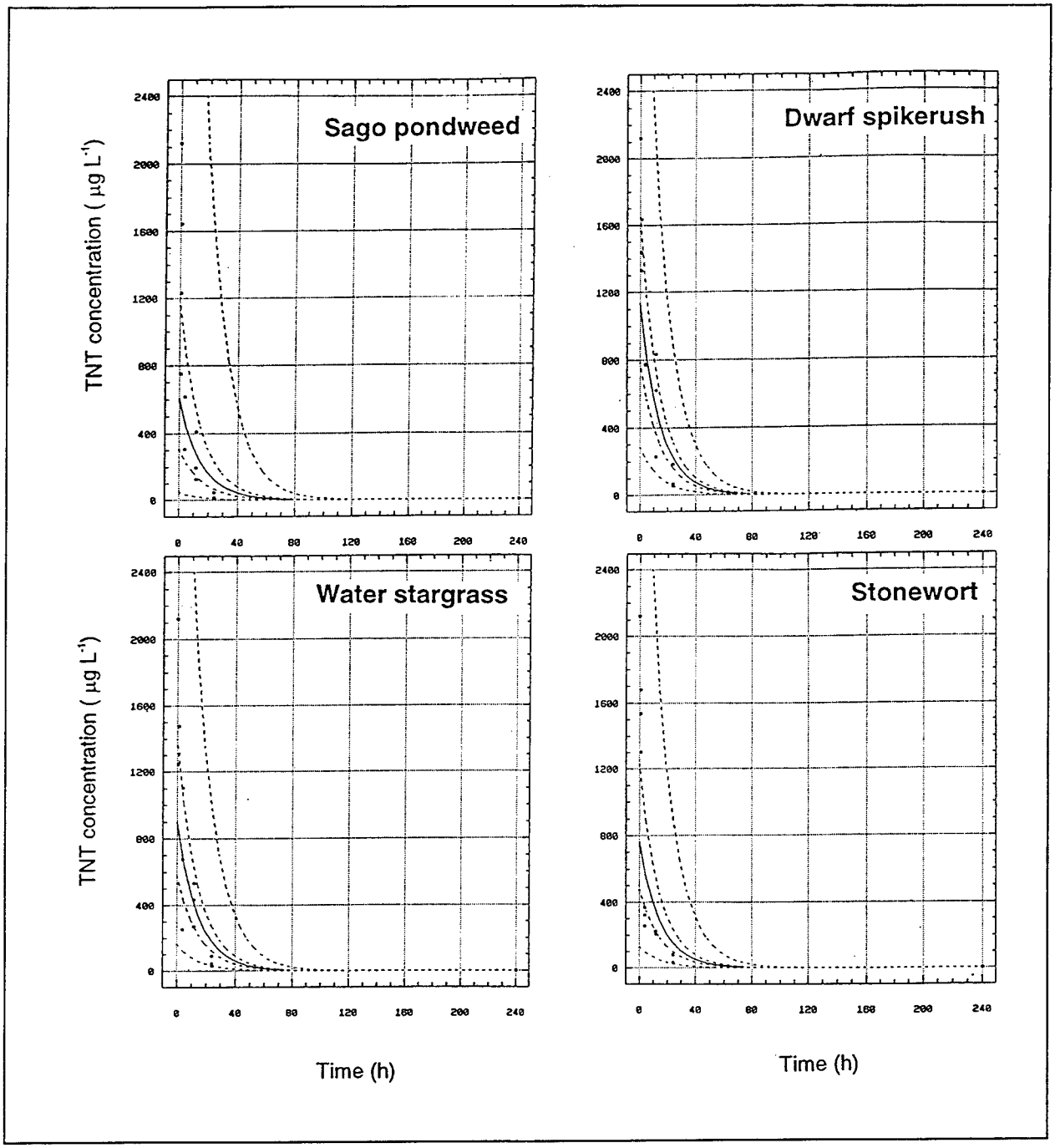

Figure 1. (Sheet 2 of 3)

The rate of removal, K, as indicated by the slope, varied. Plant treatment slopes tended to be uniform over all three factors (average = -0.034 ±0.006), with most requiring 4 to 8 days to reach 2 µg TNT L^{-1} (the hydraulic retention time). While most control slopes were much shallower, the fertilized autoclaved sediment control had the largest of any treatment, with K = -0.041, giving an estimate for retention time of 7 days compared with 56 and 55 days for groundwater and unautoclaved sediment controls without N-amendment. With fertilizer, water and

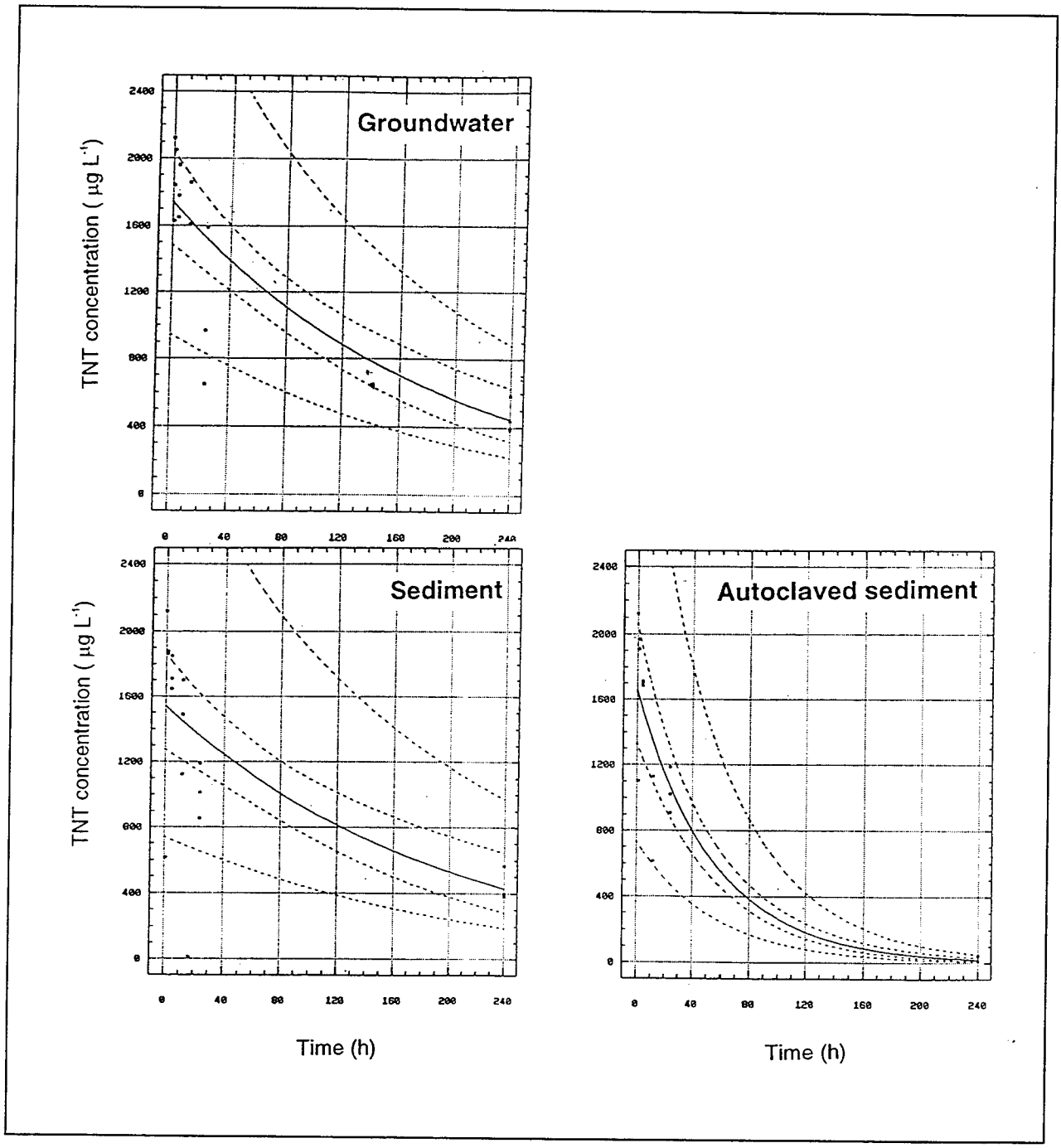

Figure 1. (Sheet 3 of 3)

sediment were more differentiated, giving 70 and 19 days to cleanup, respectively (Table 6).

Normalizing the slope of the regression curve by dividing it by the initial dry weight incubated gave the biomass-normalized removal rate *K/DW*. Comparisons emphasized that rate of removal was affected very little by plant density (K/DW_{D1}: K/DW_{D2} = 2.502, corr. coeff. = 0.930), and that plant removal rates were the same

with or without fertilization (K/DW_{D1}:K/DW_{F2} = 1.072, corr. coeff. = 0.996). However, differences among species were apparent, and they were not always similar to rankings based on changes in TNT concentration over time (Table 5). Vallisneria had the most rapid removal rate under all three factors; it may have failed to rank high in overall activity (Table 5) because its removal regressions were associated with high intercepts. Water star-grass and curlyleaf pondweed also outperformed elodea on the basis of *K/DW*. On the other hand, egeria's relative performance was low in both comparisons, associated with unusually high retention time and low *K/DW* statistics.

Table 5
Treatment Effects on TNT Concentration in Groundwater over 10-day Incubation; Multiple-Range Analysis by Treatment (Tukey's HSD): Species at Two Densities and Controls, and Species at the Lower Density with N Fertilization

Treatment	Least Significance Mean	Homogeneous Groups[1]
Lower Density - 9 g FW L^{-1}		
Elodea	630.0	a
Sago pondweed	697.4	ab
Curlyleaf p'weed	736.3	ab
Stonewort	738.2	ab
Milfoil	756.2	ab
Star-grass	764.4	ab
Parrot-feather	806.4	ab
Spikerush	849.1	abc
Vallisneria	982.9	abc
Egeria	1005.8	abc
Autocl.sediment	1263.1	abc
Sediment	1364.6	bc
Groundwater	1507.3	c
Higher Density - 18 g FW L^{-1}		
Elodea	466.4	a
Sago pondweed	532.6	a
Star-grass	548.4	a
Milfoil	585.5	ab
Parrot-feather	611.5	ab
Curlyleaf p'weed	636.0	abc
Spikerush	723.2	abc
Vallisneria	748.3	abc
Egeria	787.6	abcd
Autocl.sediment	1305.4	bcd
Sediment	1369.0	cd
Groundwater	1511.7	d

(Continued)

[1] Same letters indicate no significant differences among treatments within species.

Table 5 (Concluded)		
Treatment	Least Significance Mean	Homogeneous Groups
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1}		
Elodea	632.2	a
Stonewort	695.8	ab
Milfoil	697.0	ab
Sago pondweed	709.1	ab
Spikerush	741.3	ab
Star-grass	748.0	ab
Parrot-feather	763.0	ab
Curlyleaf p'weed	768.6	ab
Egeria	885.5	abc
Vallisneria	887.6	abc
Autocl.sediment	1199.9	abc
Sediment	1405.7	bc
Groundwater	1525.5	c

In order to assess whether TNT disappearance from water was directly mediated by plant factors such as adsoprtion and metabolic activity, initial DW biomass incubated was estimated and correlated to retention time required for cleanup (Table 6) using data for plant treatments and groundwater controls from all three treatment factors (N = 31). This negative correlation was not highly significant (corr. coeff. = -0.51, P = 0.006), but suggests that the TNT removal from water seen here had a component that was plant-mediated, and that retention time decreased with increasing DW.

Relevance of treatment effects

Comparison of treatment kinetics can identify that portion of TNT removal attributable to the presence of plants, separating it from effects of photolysis, microorganism degradation, etc., common to all incubations, or from effects related to sediment adsorption. The two components of regression--the intercepts and slopes of the fitted curves (Table 6)--can be related to the two processes of TNT removal expected to be occurring under static exposure: early rapid changes in concentration due to initial adsorption and/or uptake of explosive by plant material, sediment, or inert objects; and the steady-state change associated with biological and physical degradative processes. Some differences in adsorption among plant species can be intuitively related to morphology and consequent leaf surface area. Lower intercepts were seen in species with strap-like or cylindrical leaves (vallisneria or spikerush) than in those with dissected leaves (Eurasian watermilfoil and elodea). This initial process occurred to a lesser extent among treatments without plants whether sediment was present or not. While it has been suggested that autoclaving results in higher availability of adsorptive sites on organic matter, and may result in similar physical changes in sediment structure, increased removal with autoclaved, fertilized sediment over the other controls was associated not with decrease in intercept but with higher removal rate, $K = -0.041$.

Table 6
Curve Fit Statistics and Plant Biomass (DW)-Normalized K-Values for TNT Concentrations in Groundwater over 10-day Incubation with Plant Species at Two Densities and Controls, and with Plant Species at Lower Density and N Fertilization, Mean Mass and Standard Deviations (N = 3), Initial concentration TNT in Groundwater: 2,123 μg L^{-1}

Treatment	$Y = exp(a + bX)$			Days to 2 μg L^{-1}	K/DW	Initial Mass Incubated (g DW) Mean ± SD
	Intercept *a*	Slope *b*	R^2, %			
Lower Density - 9 g FW L^{-1}						
Parrot-feather	6.51	-0.029	70.29	8.4	-0.007	3.99 ± 0.06
Milfoil	6.24	-0.037	87.14	6.2	-0.012	3.04 ± 0.06
Egeria	7.09	-0.015	91.02	17.8	-0.004	3.32 ± 0.03
Elodea	5.94	-0.035	84.16	6.2	-0.016	2.16 ± 0.02
Vallisneria	7.17	-0.040	98.19	6.7	-0.029	1.40 ± 0.00
Curlyleaf p'weed	6.53	-0.038	92.40	6.4	-0.017	2.20 ± 0.01
Sago pondweed	6.16	-0.036	85.88	6.3	-0.016	2.21 ± 0.02
Star-grass	6.53	-0.038	92.33	6.4	-0.022	1.70 ± 0.01
Spikerush	6.80	-0.039	94.85	6.5	-0.015	2.59 ± 0.03
Stonewort	6.42	-0.037	92.24	6.4	-0.012	3.13 ± 0.06
Higher density - 18 g FW L^{-1}						
Parrot-feather	5.01	-0.033	52.21	5.5	-0.004	7.91 ± 0.02
Milfoil	5.22	-0.033	63.23	5.7	-0.006	6.00 ± 0.00
Egeria	6.62	-0.031	83.29	8.0	-0.005	6.58 ± 0.01
Elodea	3.47	-0.027	30.54	4.3	-0.006	4.32 ± 0.02
Vallisneria	6.41	-0.037	90.05	6.4	-0.013	2.78 ± 0.03
Curlyleaf p'weed	5.10	-0.033	53.12	5.6	-0.009	3.90 ± 0.07
Sago pondweed	3.33	-0.026	26.37	4.2	-0.006	4.34 ± 0.03
Star-grass	4.59	-0.031	44.89	5.2	-0.009	3.41 ± 0.04
Spikerush	5.81	-0.036	66.53	5.9	-0.007	5.15 ± 0.03
Controls						
Groundwater	7.46	-0.005	77.87	56.4		0
Sediment	7.34	-0.005	68.18	55.4		262 ± 0.95
Autocl.sediment	7.41	-0.018	95.64	15.5		256 ± 8.39
Fertilized 50 mg NO_3-N L^{-1}; 9 g FW L^{-1}						
Parrot-feather	6.03	-0.036	69.48	6.2	-0.009	4.00 ± 0.06
Milfoil	6.11	-0.037	88.12	6.1	-0.012	3.03 ± 0.10
Egeria	6.78	-0.014	73.19	18.1	-0.004	3.32 ± 0.04
Elodea	5.67	-0.035	67.70	5.9	-0.016	2.17 ± 0.03
Vallisneria	7.01	-0.039	98.08	6.7	-0.028	1.42 ± 0.01
Curlyleaf p'weed	6.60	-0.038	93.67	6.5	-0.017	2.20 ± 0.03

(Continued)

Table 6 (Concluded)

Treatment	$Y = exp\ (a + bX)$			Days to 2 $\mu g\ L^{-1}$	K/DW	Initial Mass Incubated (g DW) Mean ± SD
	Intercept *a*	Slope *b*	R^2, %			
Fertilized 50 mg NO_3-N L^{-1}; 9 g FW L^{-1} (Continued)						
Sago pondweed	5.88	-0.035	69.27	6.2	-0.016	2.17 ± 0.03
Star-grass	6.45	-0.037	90.66	6.5	-0.022	1.69 ± 0.02
Spikerush	6.57	-0.038	93.58	6.4	-0.015	2.61 ± 0.03
Stonewort	5.95	-0.036	70.40	6.1	-0.011	3.16 ± 0.08
Fertilized Controls						
Groundwater	7.44	-0.004	66.54	70.3		0
Sediment	7.51	-0.015	96.59	18.9		271 ± 0.72
Autocl.sediment	7.50	-0.041	99.15	6.9		256 ± 10.64

It is possible that the increased TNT removal in fertilizer-amended sediment may result from the ability of explosives-adapted microbial populations, expected to be present in contaminated groundwater, to function optimally when freed from competition with native sediment populations and supplied with nutrients. This type of activity would effect a change in rate.

The extrapolation of these batch evaluations to a continuous flow remediation system, where the supply of explosive is constantly renewed, suggests that final TNT concentration at 10 days as determined here is less relevant than rate of removal and retention time required to reach 2 µg TNT L^{-1}. Reduction of TNT due to adsorption to plant tissue also becomes less significant in an operating system where biomass increases relatively slowly, and a steady state of adsorption/ desorption is reached. This gives the most importance to removal rates, which show that plant-associated effects are significant, and that vallisneria has particularly high potential for remediation activity. In addition, the majority of plant treatments were very probably below 2 µg TNT L^{-1} by 48 hr. Had removal rates been based only on curve-fitting of samples taken through 24 hr, they would have been considerably higher, and it may be more realistic to base days to cleanup on this truncated regression. In comparison, estimates from control treatments showed that a 10-day residence time for water in the absence of plants or sediment would leave TNT at levels unacceptable for potable water. It is noted that, although initial adsorption of TNT to plant tissue was higher with increased biomass, plant density is not a highly manipulable factor and will be determined by the natural carrying capacity of the individual phytoremediation system.

RDX Concentration in Water: Effects of Species, Density, and Fertilization

RDX concentrations in groundwater incubated with plant material decreased significantly from initial levels of 2,934 µg L^{-1} in only a few species during the

10-day period and remained high in controls (Figure 2 and Table 7). The relatively short incubation, lack of sampling between 24 and 240 hr, and the small initial adsorption by plants probably all contributed to the higher variability seen in the RDX data.

RDX degradation by microorganisms has been found by several researchers to be minimal under aerobic conditions and to be enhanced by organic nutrients under anaerobic conditions favoring denitrification (Spanggord et al. 1980; McCormick, Cornell, and Kaplan 1984; Walsh 1990). However, a bacterial strain has been isolated recently that uses RDX as sole N-source under aerobic conditions (Binks, Nicklin, and Bruce 1995). Aerobic degradation of RDX under flask and aerobic biometer conditions has been seen to be extensive and rapid, with up to 50 percent mineralization of RDX (conversion to CO_2) in 21 days.[1]

Multiple-range analyses revealed few differences in activity among plants and controls for RDX removal (Table 8). At the lower density, none of the species lowered RDX more rapidly than the controls, while sago pondweed was the most effective species at the higher density. N-amendment enhanced the autoclaved sediment control relative to several plant species and to the groundwater control. Comparisons within species (Table 9) showed that sago pondweed, Eurasian watermilfoil, and vallisneria were more active at the higher biomass level and that fertilization did not enhance activity.

A linear regression model, $Y = a + bX$ (zero order kinetic), gave the best fit to change in RDX during incubation (Table 10). However, slopes were not uniformly negative and R^2 values were generally low as a result of variability in the data. Where slopes were negative, regression statistics were used to extrapolate residence time required to reach RDX cleanup levels, $t_{2\mu g} = [(2 - a)/b)]/24$, and plant mass-normalized K/DW. These results reflected the large variability in these data, but the comparison showed that retention times were lower and removal rates were higher with increased plant density and N-amendment. Highest rates were found in elodea and the pondweeds (Table 10).

Correlation of initial DW plant mass to required retention times was not significant (corr. coeff. 0.002, signif. level 0.994, N = 23, with negative retention times omitted), suggesting that RDX removal from water is not directly affected by plant mass. The correlation between RDX retention time and final O_2 concentration in incubation water (Figure 3) was not significant (corr. coeff. 0.43; signif. level 0.045; N = 23).

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[2] Scott Weisner, personal communication, October 1996, University of Missouri, Columbia.

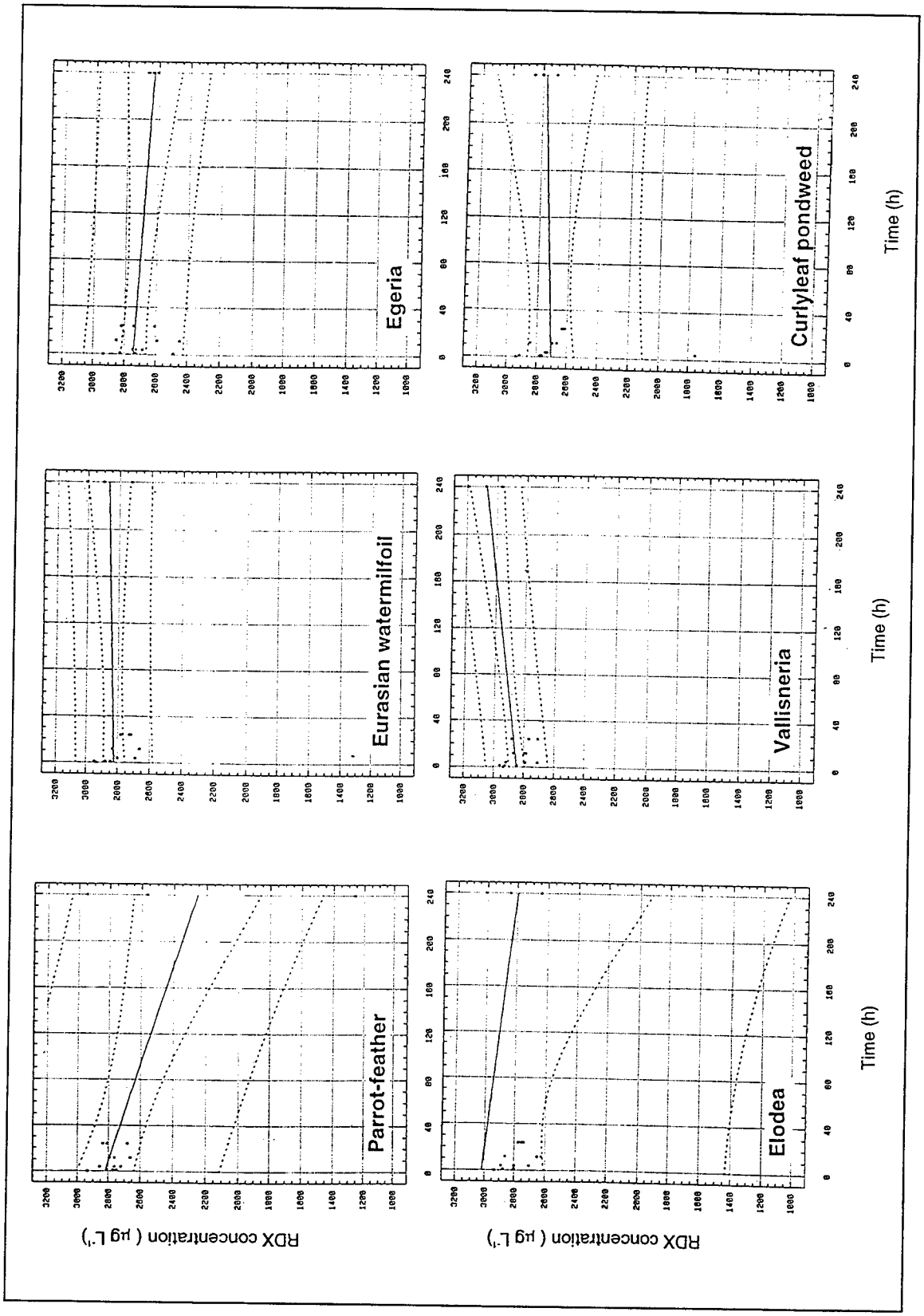

Figure 2. Changes in RDX concentration over time of groundwater incubated with one of ten aquatic plant species, alone or with unautoclaved or autoclaved sediment. Solid lines, fitted curves; interrupted lines, 95 and 90 percent confidence levels (Sheet 1 of 3)

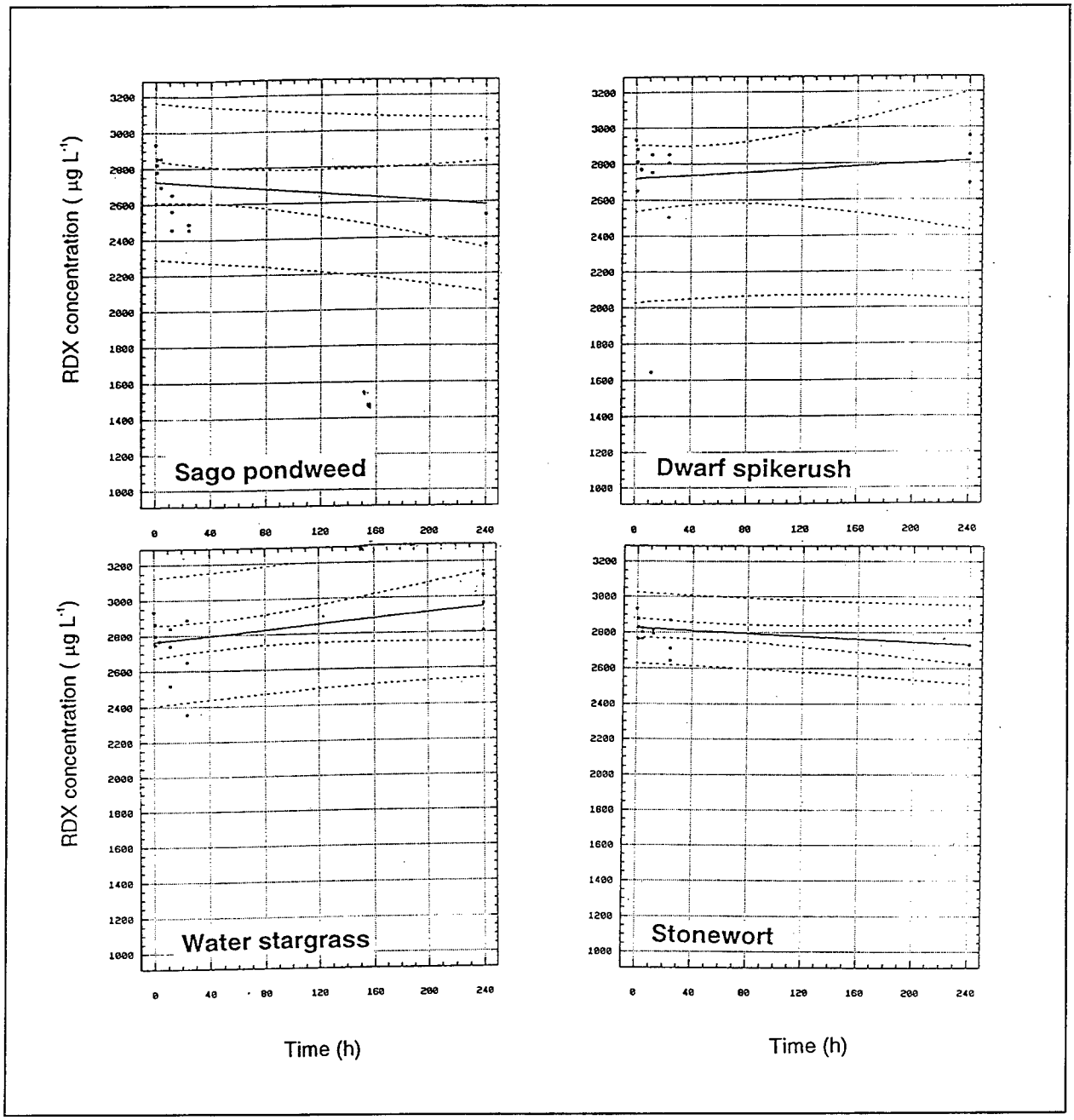

Figure 2. (Sheet 2 of 3)

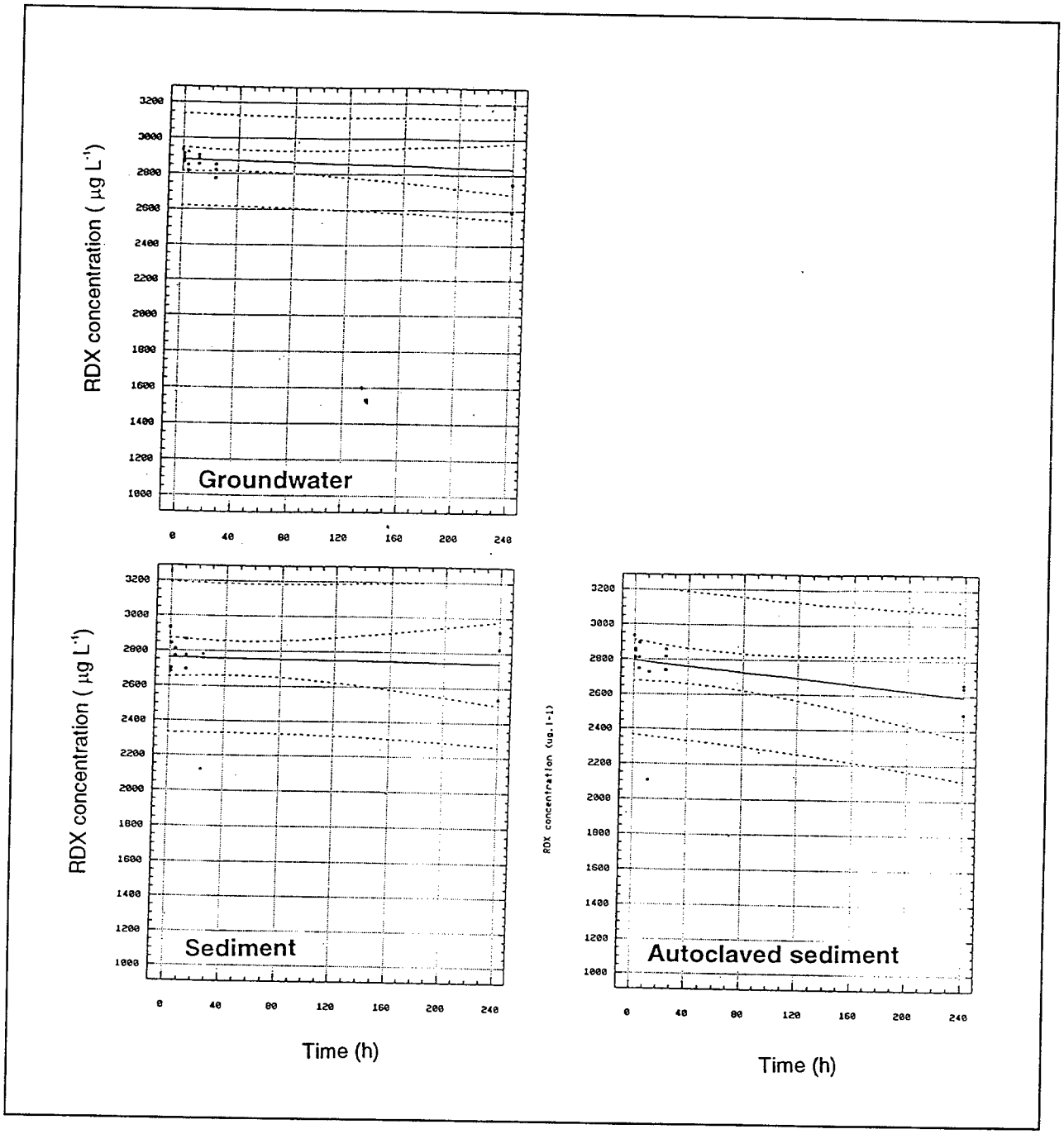

Figure 2. (Sheet 3 of 3)

Table 7
RDX Concentrations, $\mu g\ L^{-1}$, in Groundwater over 10-Day Incubation with Plant Species at Two Densities and Controls, and with Plant Species at Lower Density and N Fertilization, Mean Values (N = 3)

Treatment	Incubation Period, hr						Removal[1], %
	0	1	4	12	24	240	
Lower Density - 9 g FW L^{-1}							
Parrot-feather	2934	2777	2780	2739	2776	2257	23
Milfoil	2934	2889	2745	2746	2740	2879	2
Egeria	2934	2678	2717	2628	2723	2638	10
Elodea	2934	3813	2798	2713	2755	2836	3
Vallisneria	2934	2910	2807	2813	2787	3082	-5
Curlyleaf p'weed	2934	2817	2407	2734	2622	2789	5
Sago pondweed	2934	2820	2776	2557	2516	2613	11
Star-grass	2934	2804	2769	2699	2631	2966	-1
Spikerush	2934	2782	2772	2418	2720	2832	4
Stonewort	2934	2825	2798	2804	2742	2741	7
Higher density - 18 g FW L^{-1}							
Parrot-feather	2934	2723	2608	2775	2618	2104	28
Milfoil	2934	2720	2658	2758	2760	1648	44
Egeria	2934	2794	2752	2772	2758	2747	6
Elodea	2934	2791	2546	2623	2787	2524	14
Vallisneria	2934	2715	2654	2748	2643	2595	12
Curlyleaf p'weed	2934	2715	2705	2711	2778	2121	27
Sago pondweed	2934	2609	2714	2537	2338	97	97
Star-grass	2934	2752	2768	2734	2719	2725	7
Spikerush	2934	2752	2786	2692	2621	2642	10
Controls							
Groundwater	2934	2892	2863	2882	2817	2840	3
Sediment	2934	2741	2784	2780	2553	2759	6
Autocl.sediment	2934	2839	2818	2417	2806	2600	11
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1}							
Parrot-feather	2934	2873	2796	2745	2757	2590	22
Milfoil	2934	2812	2823	2855	2835	2655	10
Egeria	2934	2800	2799	2725	2640	2502	15
Elodea	2934	2900	2857	2743	2875	2178	26
Vallisneria	2934	2855	2792	2654	2742	2609	11
Curlyleaf p'weed	2934	2768	2767	2781	2766	2247	23
Sago pondweed	2934	2788	2757	2762	2690	2585	22
Star-grass	2934	2877	2751	2845	2794	3046	-4

(Continued)

[1] Based on concentrations at 10 days.

Table 7 (Concluded)

Treatment	Incubation Period, hr						Removal[1], %
	0	1	4	12	24	240	
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1} (Continued)							
Spikerush	2934	2720	2801	2765	2772	873	2
Stonewort	2934	2811	2884	2804	2855	2856	3
Fertilized Controls							
Groundwater	2934	2857	2793	2793	2847	3094	-5
Sediment	2934	2896	2798	2806	2555	1805	38
Autocl.sediment	2934	2814	2632	2660	2461	1395	52

Species Effects on TNT Metabolites and Degradation Products

Nitrobody removal

It has been shown that in aquatic environments several explosives, including TNT, can disappear rapidly from water due to photolysis because they are sensitive to irradiance above 290 nm (ultraviolet (UV) and visible light), and that adsorption is not significant (Spanggord et al. 1980; Gorontzy et al. 1994). TNT is commonly transformed by microorganisms to ADNTs, DANTs, and azoxy compounds in water and sediments (Walsh 1990; Spanggord et al. 1980). Van Beelen and Burris (1995) found that crude extracts from aquatic plants reduced TNT to ADNTs and DANTs under aerobic and anaerobic conditions. Plant cell cultures have been shown to use metabolic pathways of nitroreduction in combination with oxidation of the methyl group (Mueller et al. 1995). Here, metabolites of reduction pathways as well as the photolytic products of TNT were examined in groundwater and plant tissue to characterize degradation.

Several TNT reduction products were present in the initial groundwater: 2ADNT at 43 μg L^{-1}, 4ADNT at 36 μg L^{-1}, 2,4DANT at 7 μg L^{-1}, and 2,6DANT at 74 μg L^{-1} (Table 2). Changes in their individual concentrations over the incubation time course are shown in Figure 4, and TADNTs and TDANTs are combined and summarized in Tables 11 and 12, respectively. 4ADNT is the initial reduction product of TNT in many organisms (Walsh 1990; Spanggord et al. 1980). Here it was seen to increase four- to eightfold in the presence of most plant species by 24 hr, before returning to initial or lower levels at 10 days. Parrotfeather generated the highest levels of this compound among the plants, 333 ± 6.7 μg L^{-1} at 24 hr at the lower density; but at the end of incubation 4ADNT remained at the highest concentration in autoclaved sediment without fertilizer (312 ± 3.8 μg L^{-1}). Those plant incubations in which this monoamino remained ≥100 μg L^{-1} at 10 days--parrot-feather, egeria, vallisneria, and spikerush--include those found to be slower in TNT removal (Table 4). Incubation without plants generally slowed production of 4ADNT, with relatively small increases occurring after 24 hr. Fertilization slowed increase in 4ADNT in controls.

Table 8
Treatment Effects on RDX Concentration in Groundwater over 10-day Incubation; Multiple-Range Analysis by Treatment (Tukey's HSD): Species at Two Densities and Controls, and Species at the Lower Density with N Fertilization

Treatment	Least Significance Mean	Homogeneous Groups[1]
Lower Density - 9 g FW L^{-1}		
Sago pondweed	2698.7	a
Parrot-feather	2710.3	a
Curlyleaf p'weed	2717.1	a
Egeria	2719.7	a
Spikerush	2741.9	a
Autocl.sediment	2754.8	a
Sediment	2758.5	a
Star-grass	2800.5	a
Stonewort	2807.8	a
Milfoil	2832.9	a
Groundwater	2871.2	a
Vallisneria	2888.6	a
Elodea	2974.8	a
Higher density - 18 g FW L^{-1}		
Sago pondweed	2204.8	a
Milfoil	2579.3	b
Parrot-feather	2626.7	b
Curlyleaf p'weed	2661.6	b
Elodea	2700.3	b
Spikerush	2714.8	b
Vallisneria	2737.7	b
Autocl.sediment	2758.4	b
Sediment	2764.2	b
Star-grass	2771.6	b
Egeria	2792.5	b
Groundwater	2871.2	b
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1}		
Autocl.sediment	2482.2	a
Sediment	2631.8	ab
Curlyleaf p'weed	2710.1	ab

(Continued)

Note: ANOVA showed that treatment and time affected RDX concentration significantly at the higher density and at fertilization ($P<0.001$), but not at the lower density (P treatment = 0.114, P time = 0.237).

[1] Same letters indicate no significant differences among treatments within species.

Table 8 (Concluded)

Treatment	Least Significance Mean	Homogeneous Groups
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1} (Continued)		
Egeria	2733.0	ab
Elodea	2747.5	ab
Sago pondweed	2754.5	ab
Vallisneria	2763.8	b
Parrot-feather	2782.0	b
Spikerush	2810.4	b
Milfoil	2819.5	b
Stonewort	2856.8	b
Star-grass	2874.2	b
Groundwater	2886.1	b

Table 9
Treatment Effects within Species on RDX Concentration in Groundwater over 10-Day Incubation; Multiple-Range Analysis (Tukey's HSD) of Individual Species and Controls Across Levels of Density and N Fertilization Based on ANOVA of Treatment, Using Time as Covariant

Species	Treatment[1]						P-Value
	Lower Density[2]		Higher Density[3]		Fertilized[4]		
	Least Significance Mean	Homo-geneous Groups[5]	Least Significance Mean	Homo-geneous Groups[5]	Least Significance Mean	Homo-geneous Groups[5]	
Parrot-feather	2710.5	a	2627.1	a	2782.5	a	0.161
Milfoil	2836.9	b	2575.5	a	2816.8	b	0.003
Egeria	2720.0	a	2792.8	a	2733.4	a	0.270
Elodea	2974.9	a	2700.6	a	2747.9	a	0.160
Vallisneria	2888.8	b	2715.1	a	2764.3	ab	0.018
Curlyleaf p'weed	2716.3	a	2659.9	a	2709.4	a	0.829
Sago pondweed	2702.6	b	2198.9	a	2754.4	b	0.002
Water star-grass	2800.8	a	2771.9	a	2874.6	a	0.090
Spikerush	2741.4	a	2738.1	a	2810.8	a	0.501

[1] RDX concentration significantly affected by time in five species and controls ($P < 0.001$); not significantly affected in elodea, water star-grass, vallisneria, and spikerush; P-range 0.053 to 0.917).
[2] 9g FW L^{-1}
[3] 18g FW L^{-1}
[4] 50mg NO_3-N L^{-1}, 9g FW L^{-1}
[5] Same letters indicate no significant differences among treatments within species.

Table 10
Curve Fit Statistics and Plant Biomass (DW)-Normalized K-Values for RDX Concentrations in Groundwater over 10-day Incubation with Plant Species at Two Densities and Controls, and with Plant Species at Lower Density and N Fertilization, Mean Mass and Standard Deviations (N = 3), Initial Concentration RDX in Groundwater: 2,934 $\mu g\ L^{-1}$

Treatment	$Y = a + bX$			Days to 2 $\mu g\ L^{-1}$	*K/DW*	Initial Mass Incubated (g DW) Mean ± SD
	Intercept *a*	Slope *b*	R^2 %			
Lower Density - 9 g FW L^{-1}						
Parrot-feather	2820	-2.357	31.21	50	-0.591	3.99 ± 0.06
Milfoil	2822	0.186	2.68	-	-	3.04 ± 0.06
Egeria	2741	-0.458	7.88	249	-0.138	3.32 ± 0.03
Elodea	3018	-0.920	1.37	137	-0.426	2.16 ± 0.02
Vallisneria	2844	0.943	46.14	-	-	1.40 ± 0.00
Curlyleaf p'weed	2702	0.309	1.09	-	-	2.20 ± 0.01
Sago pondweed	2727	-0.590	7.31	192	-0.267	2.21 ± 0.02
Star-grass	2765	0.758	15.34	-	-	1.70 ± 0.01
Spikerush	2722	0.393	1.38	-	-	2.59 ± 0.03
Stonewort	2826	-0.395	14.87	298	-0.126	3.13 ± 0.06
Higher Density - 18 g FW L^{-1}						
Parrot-feather	2755	-2.740	57.45	42	-0.346	7.91 ± 0.02
Milfoil	2804	-4.796	88.42	24	-0.799	6.00 ± 0.00
Egeria	2805	-0.272	5.27	429	-0.041	6.58 ± 0.01
Elodea	2743	-0.926	10.25	123	-0.231	4.32 ± 0.02
Vallisneria	2747	-0.672	12.89	170	-0.242	2.78 ± 0.03
Curlyleaf p'weed	2794	-2.798	72.37	42	-0.717	3.90 ± 0.07
Sago pondweed	2719	-10.985	95.72	10	-2.531	4.34 ± 0.03
Star-grass	2785	-0.289	4.35	401	-0.085	3.41 ± 0.04
Spikerush	2765	-0.577	12.94	200	-0.112	5.15 ± 0.03
Controls						
Groundwater	2880	-0.191	2.18	628		0
Sediment	2763	-0.099	0.22	1162		262 ± 0.95
Autocl.sediment	2796	-0.853	14.63	136		256 ± 8.39
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1}						
Parrot-feather	2831	-1.043	37.47	113	-0.261	4.00 ± 0.06
Milfoil	2858	-0.852	43.05	140	-0.281	3.03 ± 0.10
Egeria	2793	-1.274	34.69	91	-0.384	3.32 ± 0.04
Elodea	2885	-2.946	72.80	41	-1.358	2.17 ± 0.03
Vallisneria	2804	-0.861	10.66	136	-0.606	1.42 ± 0.01
Curlyleaf p'weed	2823	-2.410	29.02	49	-1.095	2.20 ± 0.03
Sago pondweed	2797	-0.929	32.86	125	-0.428	2.17 ± 0.03

(Continued)

Table 10 (Concluded)

Treatment	$Y = a + bX$			Days to 2 μg L^{-1}	K/DW	Initial Mass Incubated (g DW) Mean ± SD
	Intercept a	Slope b	R^2 %			
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1} (Continued)						
Star-grass	2834	0.848	31.86	-	-	1.69 ± 0.02
Spikerush	2797	0.293	4.54	-	-	2.61 ± 0.03
Stonewort	2858	-0.023	0.02	5174	-0.007	3.16 ± 0.08
Fertilized Controls						
Groundwater	2837	1.049	41.69	-		0
Sediment	2836	-4.368	80.35	27		271 ± 0.72
Autocl.sediment	2750	-5.722	79.66	20		256 ± 10.64

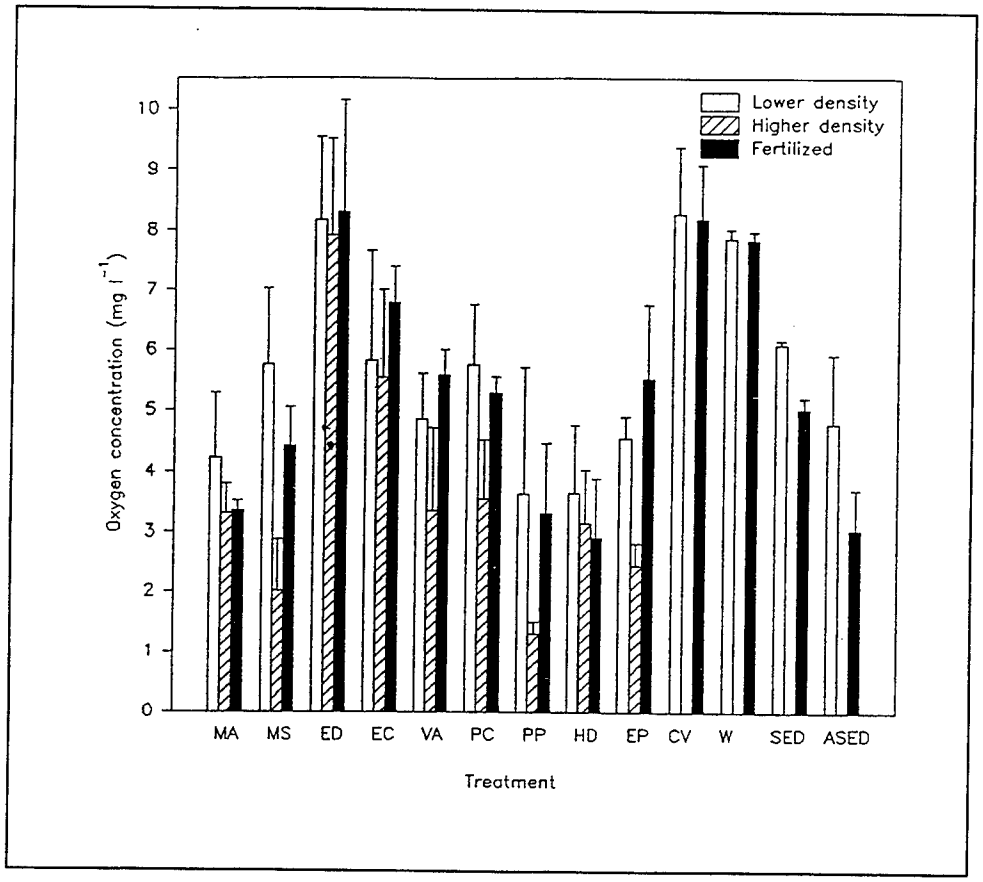

Figure 3. Oxygen concentrations in explosives-contaminated groundwater following 10-day incubation with plants, groundwater alone, or groundwater with unautoclaved or autoclaved sediment. Mean values and standard deviations (N=3). Abbreviations of plant names are defined in Appendix D.

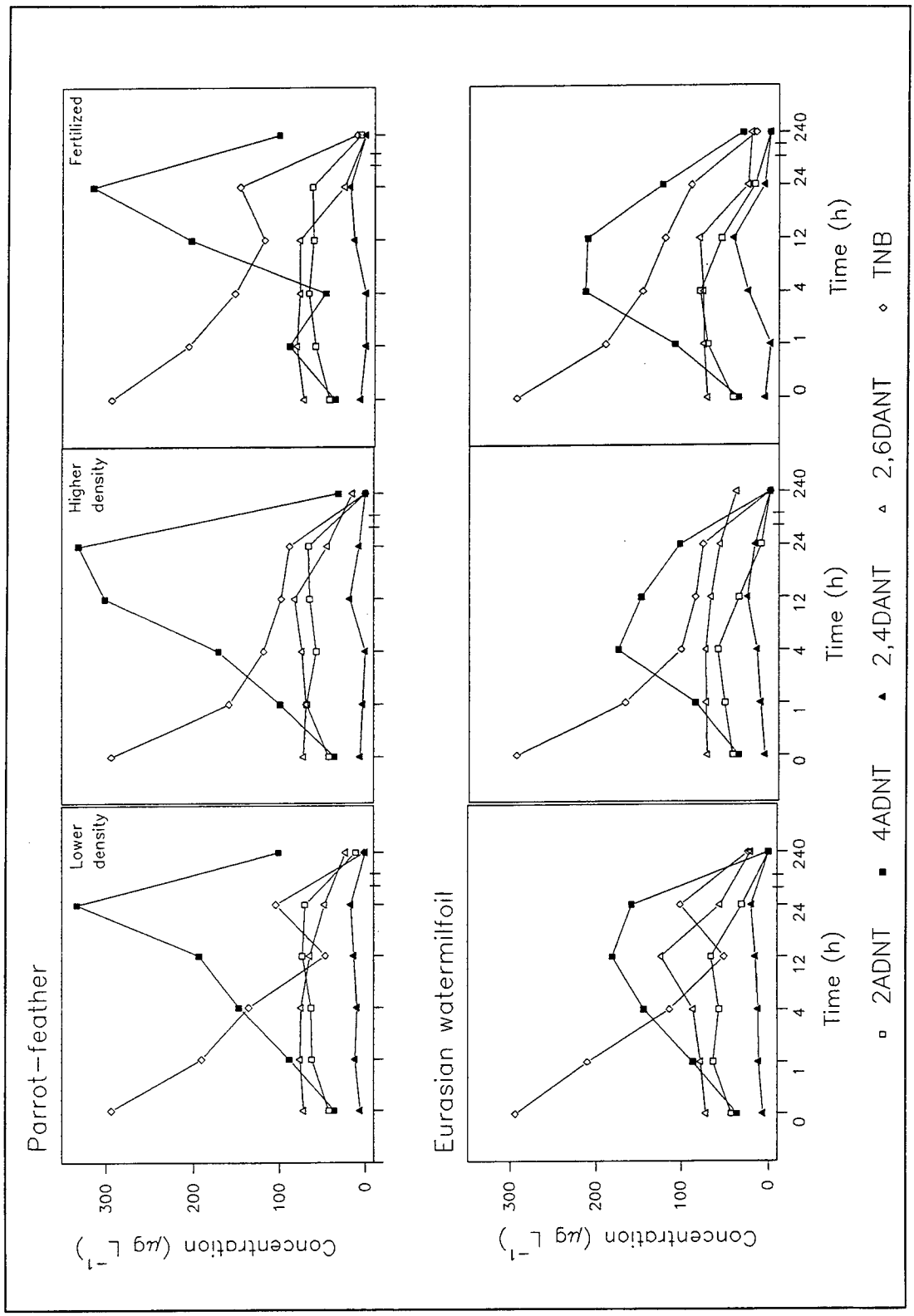

Figure 4. Concentrations of reduction and photolytic products of TNT in groundwater incubated with plants or controls at low (9 g FW L^{-1}) or high (18 g FW L^{-1}) plant density or fertilization (50 mg NO_3-N L^{-1} with 9 g FW L^{-1}). Once-reduced products: 2ADNT, 4ADNT. Twice-reduced: 2,4DANT, 2,6DANT. Photolytic product: TNB. Mean values (N = 3) (Sheet 1 of 6)

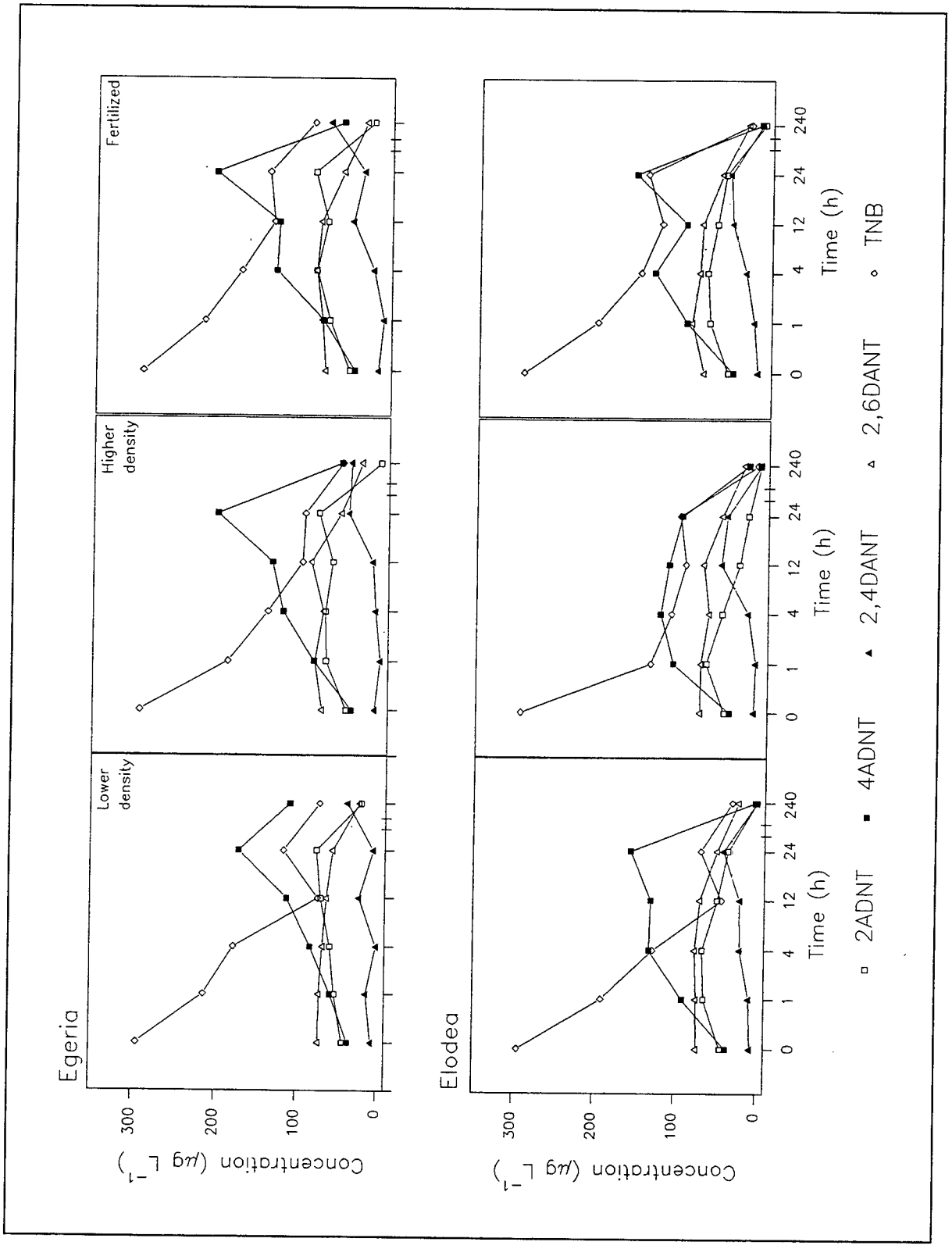

Figure 4. (Sheet 2 of 6)

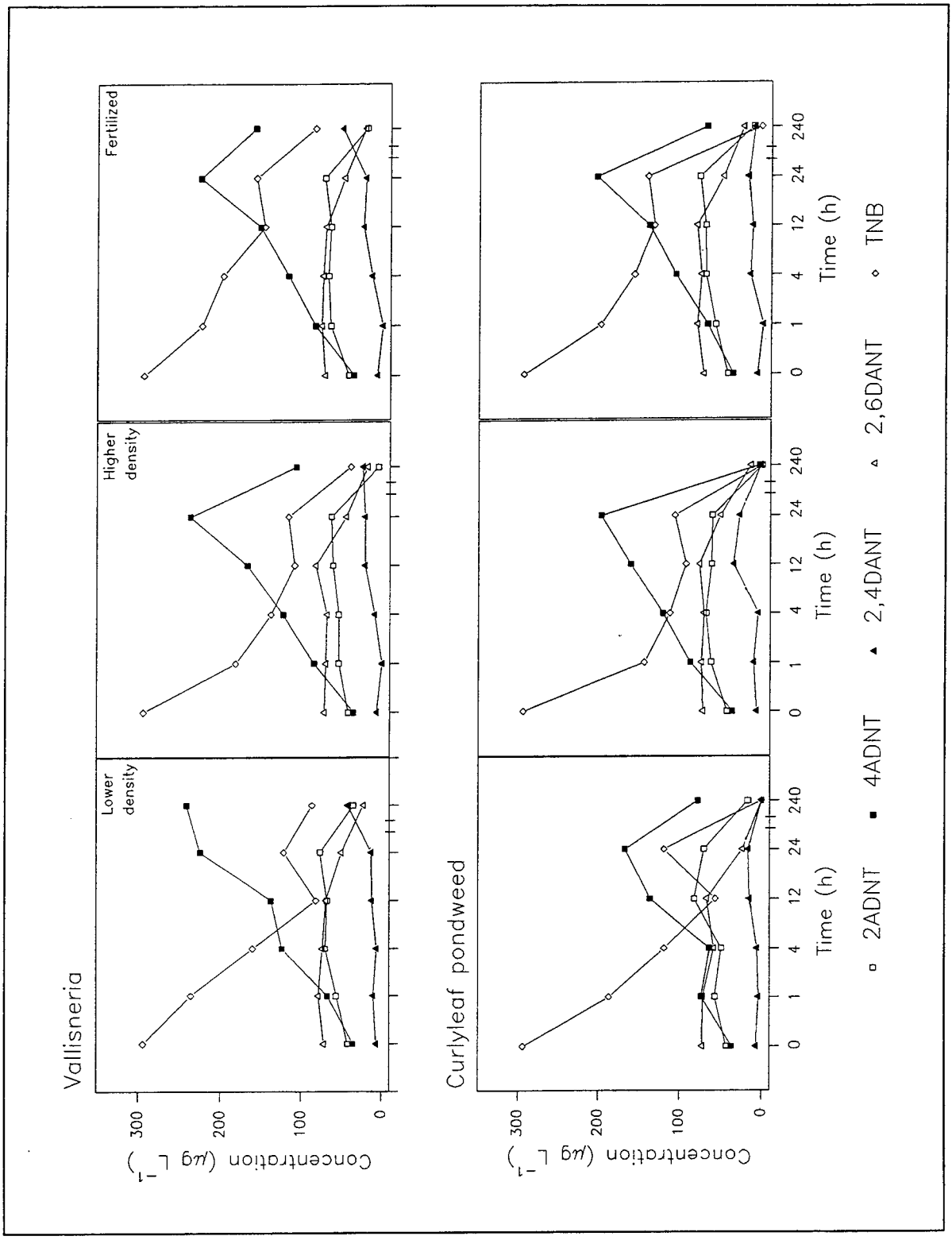

Figure 4. (Sheet 3 of 6)

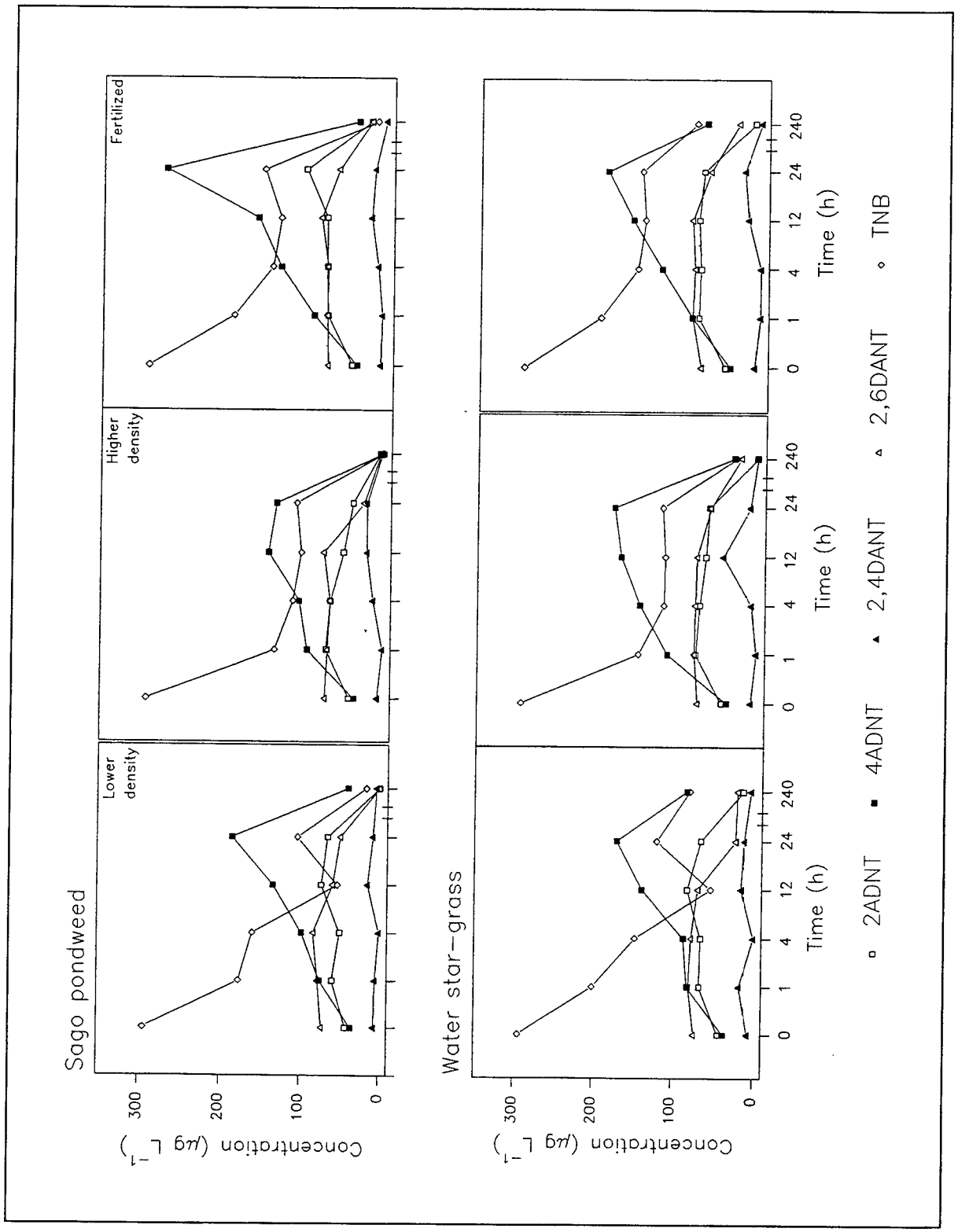

Figure 4. (Sheet 4 of 6)

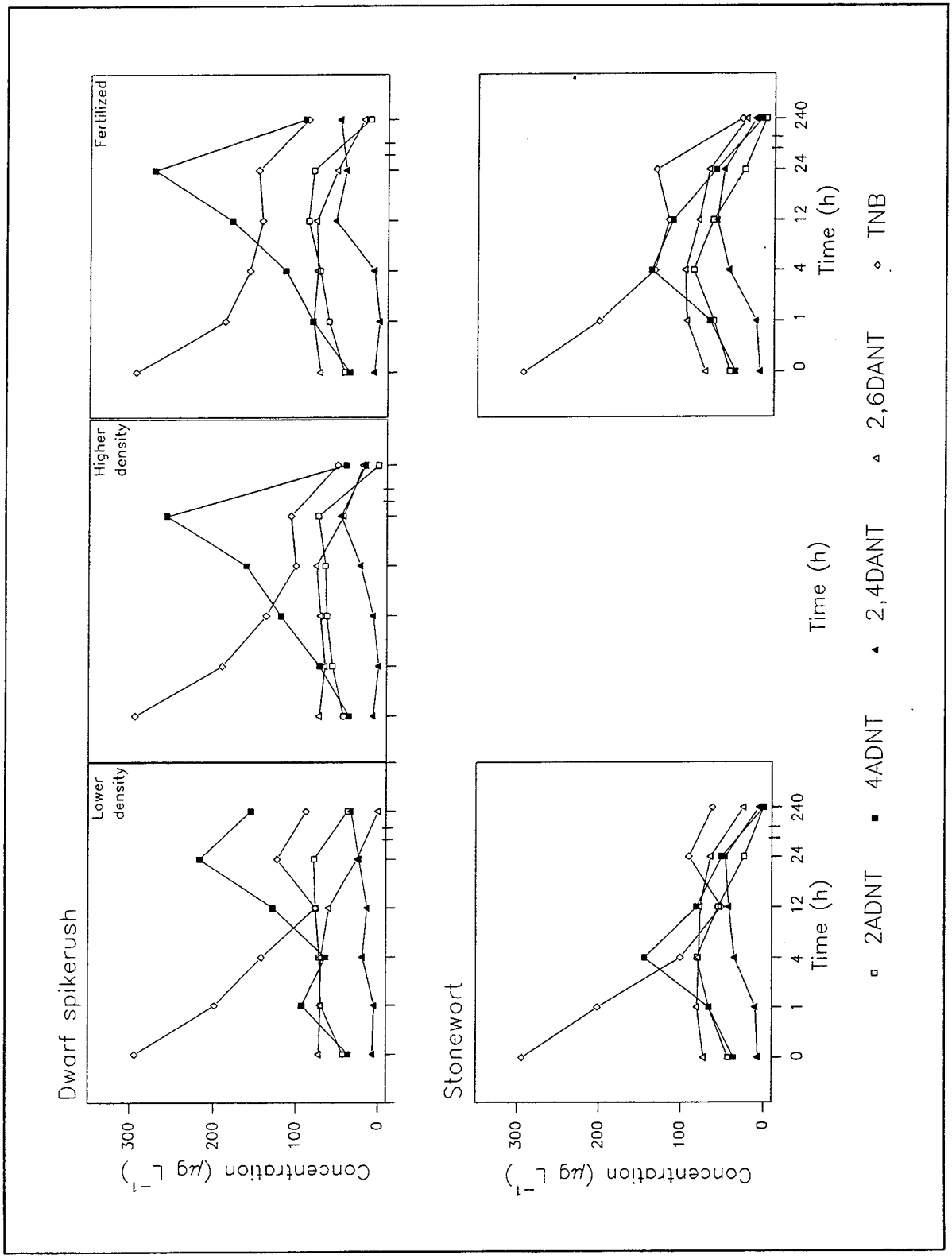

Figure 4. (Sheet 5 of 6)

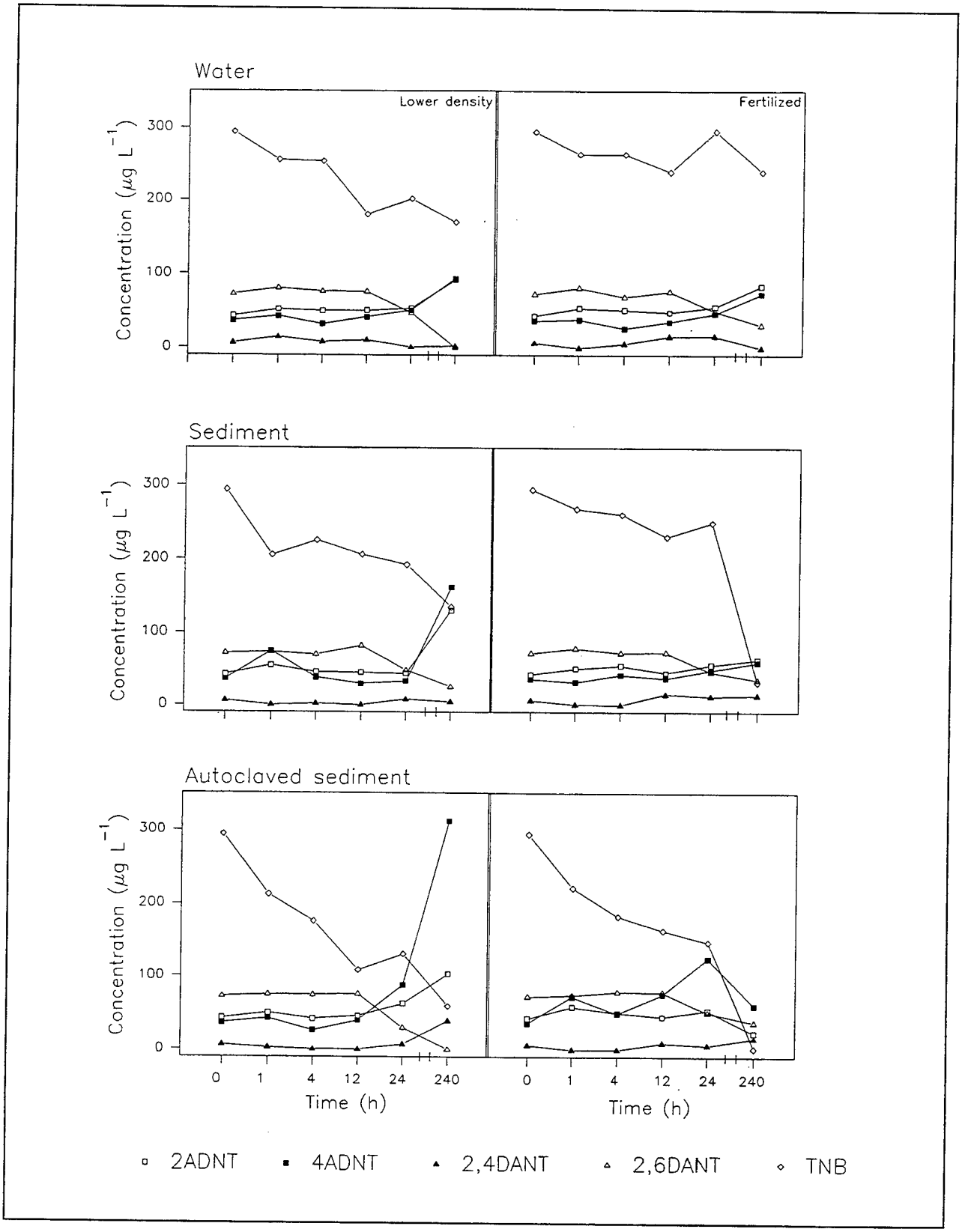

Figure 4. (Sheet 6 of 6)

Table 11
Total ADNT Concentrations, $\mu g\ L^{-1}$ in Groundwater over 10-Day Incubation with Plant Species at Two Densities and Controls, and with Plant Species at Lower Density and N Fertilization, Means Values (N = 3)

Treatment	Incubation Period, hr						Removal[1], %
	0	1	4	12	24	240	
Lower Density - 9 g FW L^{-1}							
Parrot-feather	78	151	211	267	403	113	-44
Milfoil	78	150	199	246	188	–[2]	100
Egeria	78	110	141	182	247	129	-65
Elodea	78	153	196	176	188	2	97
Vallisneria	78	124	194	205	302	277	-253
Curlyleaf p'weed	78	129	111	217	237	93	-19
Sago pondweed	78	134	148	207	250	40	49
Star-grass	78	147	150	219	234	95	-21
Spikerush	78	162	135	204	295	192	-145
Stonewort	78	131	224	134	73	–[2]	100
Higher Density - 18 g FW L^{-1}							
Parrot-feather	78	167	228	366	397	32	59
Milfoil	78	137	235	184	114	–[2]	100
Egeria	78	149	189	193	278	49	37
Elodea	78	169	166	136	109	14	82
Vallisneria	78	138	177	229	301	111	-42
Curlyleaf p'weed	78	149	189	222	257	3	96
Sago pondweed	78	166	171	194	172	4	95
Star-grass	78	184	213	231	235	28	64
Spikerush	78	128	182	226	330	40	48
Controls							
Groundwater	78	94	81	91	102	184	-135
Sediment	78	130	84	75	77	292	-272
Autocl.sediment	78	92	68	85	150	415	-429
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1}							
Parrot-feather	78	148	114	264	377	107	-37
Milfoil	78	181	295	271	229	–[2]	100
Egeria	78	142	217	200	293	65	17
Elodea	78	157	200	151	203	4	95
Vallisneria	78	149	186	217	298	178	-128
Curlyleaf p'weed	78	125	176	209	280	80	-2
Sago pondweed	78	163	206	235	375	54	31

(Continued)

[1] Based on concentrations at 10 days.
[2] $<0.1\ \mu g\ L^{-1}$.

Table 11 (Concluded)

Treatment	Incubation Period, hr						Removal[1], %
	0	1	4	12	24	240	
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1} (Continued)							
Star-grass	78	158	194	233	259	73	6
Spikerush	78	143	187	266	353	103	-32
Stonewort	78	129	225	175	85	5	94
Fertilized Controls							
Groundwater	78	91	76	82	101	156	-100
Sediment	78	83	97	82	105	123	-58
Autocl.sediment	78	130	99	120	179	83	-6

In incubations with plants, the monoamino isomer 4ADNT increased in groundwater only slightly from its initial level and decreased below it by 10 days. With sediment, however, it increased threefold during this time. This suggests that 4ADNT was the initial metabolite in the presence of these plants and of sediments. Harvey et al. (1990) also observed formation of ADNTs in hydroponic culture of bush bean with TNT, but not in controls without plants. Here, ADNTs increased most significantly in controls where sediment was present.

The diamino-nitrotoluenes decreased steadily in groundwater incubated with most plant species; 2,4DANT increased six- to sevenfold above initial levels by 10 days with egeria, vallisneria, and spikerush, and with autoclaved sediment. 2,4DNT, a carcinogen, was not detected in the original groundwater (Table 2) and was also not found during the incubation time course.

The most abundant photolysis product of TNT, TNB (Walsh 1990), was initially present at a concentration of 294 μg L^{-1} in groundwater; it decreased following incubation with plants or autoclaved sediment by 70 to 100 percent (Figure 4 and Table 13). The response of TDNBs (1,4DNB, 1,3DNB; Table 14) and NB (Table 15), suggests that few photolytic products were being produced in light. At 12 hr of incubation 1,4DNB had increased from 0 to approximately 80 μg L^{-1} in water and most plant incubations, but it disappeared at the end of 10 days. The slight increases seen in 1,3DNB and NB did not persist. The lack of DNT or NT formation during the incubations (Tables 15 and 16) is evidence that no nitro-group removal took place in the metabolism associated with plants or controls. Nitrobody removal is compared in Table 15.

Comparison of plant species effects by principal components

A PCA was used to group species with similar metabolic responses as denoted by concentrations of TNT and TNT metabolites and degradation products, and of RDX, present in the final 10-day sample from the lower density incubations (Table 16). The PCA summarized variability in these data on a series of

Table 12
Total DANT Concentrations, $\mu g\ L^{-1}$, in Groundwater over 10-Day Incubation with Plant Species at Two Densities and Controls, and with Plant Species at Lower Density and N Fertilization, Mean Values (N = 3).

Treatment	Incubation Period, hr						Removal[1], %
	0	1	4	12	24	240	
Lower Density - 9 g FW L^{-1}							
Parrot-feather	78	89	85	78	65	23	70
Milfoil	78	89	99	138	75	21	74
Egeria	78	85	68	85	60	61	23
Elodea	78	81	93	88	87	22	72
Vallisneria	78	89	80	82	64	66	17
Curlyleaf p'weed	78	75	63	81	39	–[2]	100
Sago pondweed	78	82	83	74	57	4	95
Star-grass	78	96	76	83	33	23	70
Spikerush	78	75	88	73	48	33	58
Stonewort	78	90	112	117	109	29	63
Higher Density - 18 g FW L^{-1}							
Parrot-feather	78	71	74	100	52	15	79
Milfoil	78	84	89	94	75	38	51
Egeria	78	81	76	96	90	61	22
Elodea	78	76	75	115	85	19	76
Vallisneria	78	70	78	104	66	42	46
Curlyleaf p'weed	78	84	75	110	78	15	81
Sago pondweed	79	69	79	94	44	5	94
Star-grass	78	76	82	114	65	20	74
Spikerush	78	65	77	97	88	35	55
Controls							
Groundwater	78	94	83	85	47	2	98
Sediment	78	74	72	82	56	30	62
Autocl.sediment	78	77	74	75	37	39	50
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1}							
Parrot-feather	78	81	77	91	44	–[2]	100
Milfoil	78	76	103	124	73	21	73
Egeria	78	76	96	117	76	89	-14
Elodea	78	98	98	112	91	22	72
Vallisneria	78	76	88	95	69	72	8
Curlyleaf p'weed	78	80	90	93	66	34	66

(Continued)

[1] Based on concentrations at 10 days.
[2] $<0.1\ \mu g\ L^{-1}$.

Table 12 (Concluded)

Treatment	Incubation Period, hr						Removal[1], %
	0	1	4	12	24	240	
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1} (Continued)							
Sago pondweed	78	79	83	100	74	19	76
Star-grass	78	83	80	98	81	27	65
Spikerush	78	81	82	130	92	68	13
Stonewort	78	106	141	138	117	35	55
Fertilized Controls							
Groundwater	78	80	74	91	65	31	60
Sediment	78	80	73	88	58	49	37
Autocl.sediment	78	74	79	88	57	53	32

independent axes in multidimensional space, showing that 45 percent of variation was associated with the first two component axes. The spatial relationship among treatments on these axes (Figure 5) indicates similarities among type and quantity of explosives and breakdown products remaining at the end of incubation, and, by inference, similarities in physiology or kinetics of metabolism.

By plotting each replication within treatments it is clear that there was strong similarity in plant activity associated with Component 1, and that within species variability was greatest along the axis of Component 2. Water and unautoclaved sediment treatments are associated together, in contrast to plant and autoclaved sediment treatments. Autoclaved sediment has a response more similar to those species (egeria, vallisneria, and spikerush) that ranked lower in the multiple-range tests (Table 5) and had less negative regression slopes (Table 6). By inspection (Table 16), the first axis groups largely on the basis of final TNT concentration, as the high variability among treatments in this parameter is expected to be the main component of the first component. Both plant and control treatments are contrasted on the second axis, and this component differentiates largely on the basis of concentrations of 2ADNT, 4ADNT, and TNB.

The separation of the autoclaved sediment from the other controls suggests that these two groups represent dissimilar kinetics of explosives removal. This may be caused by the increased adsorption that is expected to result from autoclaving or by an unknown enhancement of microbial biotransformation by this process.

Explosives and TNT Metabolites in Plant Tissues and Sediment

Concentration levels

Plant tissue and control sediments sampled at the end of the incubation period underwent HPLC analysis to assess the ultimate fate of explosives. Unincubated

Table 13
TNB Concentrations, $\mu g\ L^{-1}$, in Groundwater over 10-Day Incubation with Plant Species at Two Densities and Controls, and with Plant Species at Lower Density and N Fertilization

Treatment	Incubation Period, hr						Removal[1], %
	0	1	4	12	24	240	
Lower Density - 9 g FW L^{-1}							
Parrot-feather	294	191	136	47	105	1	100
Milfoil	294	210	113	51	101	23	92
Egeria	294	214	178	74	116	72	76
Elodea	294	190	126	42	67	29	90
Vallisneria	294	236	160	82	122	87	71
Curlyleaf p'weed	294	187	119	56	119	–[2]	100
Sago pondweed	294	177	159	53	103	17	94
Star-grass	294	200	146	52	120	78	73
Spikerush	294	199	142	76	122	88	70
Stonewort	294	202	100	51	90	61	79
Higher Density - 18 g FW L^{-1}							
Parrot-feather	294	159	119	98	88	–[2]	100
Milfoil	294	167	102	86	77	–[2]	100
Egeria	294	188	139	97	94	48	84
Elodea	294	133	108	90	97	4	99
Vallisneria	294	182	138	109	116	39	87
Curlyleaf p'weed	294	144	113	93	106	–[2]	100
Sago pondweed	294	136	113	103	109	–[2]	100
Star-grass	294	146	114	113	116	26	92
Spikerush	294	190	137	101	107	50	83
Controls							
Groundwater	294	255	253	180	201	169	42
Sediment	294	205	226	206	192	135	54
Autocl.sediment	294	212	176	108	131	59	80
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1}							
Parrot-feather	294	207	153	118	147	10	97
Milfoil	294	191	147	122	130	15	95
Egeria	294	221	176	136	142	88	70
Elodea	294	203	150	124	142	16	95
Vallisneria	294	224	198	147	157	84	71
Curlyleaf p'weed	294	198	157	133	141	2	99
Sago pondweed	294	190	142	132	153	11	96

(Continued)

[1] Based on concentrations at 10 days.
[2] $<0.1\ \mu g\ L^{-1}$.

Table 13 (Concluded)

Treatment	Incubation Period, hr						Removal[1], %
	0	1	4	12	24	240	
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1} (Continued)							
Star-grass	294	198	151	142	146	79	73
Spikerush	294	188	158	143	148	87	70
Stonewort	294	202	134	117	132	29	90
Fertilized Controls							
Groundwater	294	263	263	238	295	238	19
Sediment	294	268	261	230	250	32	89
Autocl.sediment	294	222	183	164	148	2	99

plant material of each species (a nonexposed reference) was also analyzed to assess whether naturally occurring biochemical constituents of the plants co-eluted with target contaminants. The resulting extracts were all below detection levels of TNT. This lack of accumulation in plant tissue was consistent with findings from similar plant remediation screenings (Best et al. 1997). Metabolites and photolytic products of this explosive were generally low in plant tissue and approximately at detection level in sediments (Figure 6). The only product of single reduction of TNT was 4ADNT, although in water both ADNTs and both DANTs were present. TNT derivatives with nitro-groups removed (DNTs, NTs) were absent from plants and sediments.

Cataldo et al. (1989) found that TNT and ADNT were root-absorbed from hydroponic solution and that the majority of extractable metabolites in plant tissues were ADNT isomers. While Van Beelen and Burris (1995) found that crude extracts from aquatic plants reduced TNT to ADNTs and DANTs under aerobic and anaerobic conditions, the presence of only 4ADNT in plants here is consistent with the excess of 4ADNT over 2ADNT found in *Cyperus esculentus* L. by Palazzo and Leggett (1986). Pennington (1988) found no TNT or 2ADNT uptake in the same species. While terrestrial plants have been seen to accumulate ^{14}C-TNT-derived label (Fellows, Harvey, and Cataldo 1995), the current study identified 4ADNT only, and not TNT, in plant tissue. Taken with the data from the incubated water, this suggests that 4ADNT is an initial product of a TNT degradative pathway occurring in these species, as in microbial biotransformations of TNT (Walsh 1990).

The absence of 2,6DANT and 2,4DANT in plant tissue, despite the presence of these metabolites in incubation water, is noted. The presence of substantial amounts of the photolytic product TNB in plants suggests that this compound is readily absorbed. The absence of products derived from TNT by removal of one or more nitro-groups (DNTs and NTs) points out that no alternate pathways for further degradation of the TNT molecule occurred in these plant species.

Table 14
TDNB Concentrations, $\mu g\ L^{-1}$, in Groundwater over 10-Day Incubation with Plant Species at Two Densities and Controls, and with Plant Species at Lower Density and N Fertilization

Treatment	Incubation Period, hr						Removal[1], %
	0	1	4	12	24	240	
Lower Density -9 g FW L^{-1}							
Parrot-feather	18	28	21	75	8	–[2]	100
Milfoil	18	27	16	85	7	–[2]	100
Egeria	18	28	23	86	29	–[2]	100
Elodea	18	29	21	86	10	4	78
Vallisneria	18	34	19	50	13	–[2]	100
Curlyleaf p'weed	18	25	19	84	11	–[2]	100
Sago pondweed	18	32	43	88	10	–[2]	100
Star-grass	18	29	20	94	13	–[2]	100
Spikerush	18	28	20	82	9	13	28
Stonewort	18	28	11	78	–[2]	–[2]	100
Higher Density - 18 g FW L^{-1}							
Parrot-feather	18	31	24	23	23	–[2]	100
Milfoil	18	21	18	12	–[2]	–[2]	100
Egeria	18	27	19	17	7	–[2]	100
Elodea	18	17	19	13	–[2]	–[2]	100
Vallisneria	18	27	19	22	12	2	89
Curlyleaf p'weed	18	21	22	18	8	–[2]	100
Sago pondweed	18	23	20	16	25	–[2]	100
Star-grass	18	28	21	29	5	–[2]	100
Spikerush	18	29	23	15	8	–[2]	100
Controls							
Groundwater	18	32	25	99	9	10	44
Sediment	18	26	22	26	42	32	-178
Autocl.sediment	18	21	11	16	11	3	83
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1}							
Parrot-feather	18	34	31	32	26	–[2]	100
Milfoil	18	47	33	35	15	–[2]	100
Egeria	18	35	37	39	21	–[2]	100
Elodea	18	43	41	33	58	–[2]	100
Vallisneria	18	31	36	37	61	3	83
Curlyleaf p'weed	18	36	36	41	24	–[2]	100
Sago pondweed	18	38	33	37	42	–[2]	100

(Continued)

[1] Based on concentrations at 10 days.
[2] $<0.1\ \mu g\ L^{-1}$.

Table 14 (Concluded)

Treatment	Incubation Period, hr						Removal[1], %
	0	1	4	12	24	240	
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1} (Continued)							
Star-grass	18	33	35	37	25	9	50
Spikerush	18	35	34	34	19	13	28
Stonewort	18	33	32	30	11	–[2]	100
Fertilized Controls							
Groundwater	18	37	34	40	34	20	-11
Sediment	18	38	45	36	27	–[2]	100
Autocl.sediment	18	35	34	38	18	–[2]	100

Biotransformation

The generally low TNT metabolite concentrations in plant material (Figure 6), versus those in the incubation water with or without sediment, suggest that either most TNT degradation occurs in the plants early in the incubation period, followed by TNT metabolite leaching from the plants to the water, or that most TNT degradation occurs outside the plants via microorganism activity in water and is stimulated by plant leachates. A combination of both activities is possible. Potentially two TNT reductive mechanisms, either biotic or chemical, were operating in the incubation water with or without sediment, but only one in the plants at 10 days of incubation. No pathway involving the removal of one or more nitro-groups from TNT was detected.

The concentration of RDX was substantial in all plant parts in contact with water, but was highest in the aerial portion of parrot-feather, approximately 200 μg g^{-1} DW (Figure 6). This tissue concentration of 1,181 μg L^{-1} in extract compares to 2,257 μg L^{-1} in the groundwater incubated with the plant at 10 days. Thus, although not bioconcentrated in plants above levels found in the culture solution, RDX was taken up and possibly transported in tissue. Similar high levels of RDX relative to TNT in plants have been seen in other studies (Cataldo, Harvey, and Fellows 1990; Harvey et al. 1991; Best et al. 1997). High RDX concentrations in aerial plant portions not in direct contact with water may have originated from uptake and transport or from condensation and crystal formation on the exterior of the plants.

Azoxy Compounds

Azoxy compounds are secondary TNT degradation products that may be generated by spontaneous intermolecular condensation of nitroso- and hydroxylamino intermediates (Rieger and Knackmuss 1995). No azoxy compounds were found in the 10-day water samples analyzed from a single block, despite the pH of 5.4 to

Table 15
Percent Removal of Nitrobodies Based on 10-Day Levels for the Incubations, Means of Triplicates

Treatment	Removal of Nitrobodies								Number of Nitrobodies with >80% Removal
	TNT	RDX	TNB	TADNT	TDANT	TDNT	TDNB	NB	
Initial Concentration	2,123	2,934	294	78	78	<0.1	18	<0.1	
Lower Density - 9 g FW L^{-1}									
Parrot-feather	99	23	100	-44	70	0	100	0	3
Milfoil	100	2	92	100	74	0	100	0	4
Egeria	98	10	76	-65	23	0	100	0	2
Elodea	100	3	90	97	72	0	78	0	3
Vallisneria	100	5	71	-253	17	0	100	0	2
Curlyleaf p'weed	100	5	100	-19	100	0	100	0	4
Sago pondweed	100	11	94	49	95	0	100	0	4
Star-grass	100	-1	73	-21	70	0	100	0	2
Spikerush	100	4	70	-145	58	0	28	0	1
Stonewort	100	7	79	100	63	0	100	0	3
Higher Density - 18 g FW L^{-1}									
Parrot-feather	99	28	59	59	81	0	100	0	3
Milfoil	100	44	100	100	51	0	100	0	4
Egeria	100	6	84	37	22	0	100	0	3
Elodea	100	14	99	82	76	0	100	0	4
Vallisneria	100	12	87	-42	46	0	89	0	3
Curlyleaf p'weed	100	27	100	96	81	0	100	0	5
Sago pondweed	100	97	100	95	94	0	100	0	6
Star-grass	100	7	92	64	74	0	92	0	3
Spikerush	100	10	83	48	55	0	83	0	3
Controls									
Groundwater	78	3	42	-135	98	0	44	0	1
Sediment	79	6	54	-272	62	0	-178	0	0
Autocl.sediment	99	11	80	-429	50	0	83	0	3
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1}									
Parrot-feather	100	22	97	-37	100	0	100	0	4
Milfoil	100	10	95	100	73	0	100	0	4
Egeria	98	15	70	17	-14	0	100	0	2
Elodea	100	26	95	95	72	0	100	0	4
Vallisneria	100	11	71	-128	8	0	83	0	2
Curlyleaf p'weed	100	23	99	-2	66	0	100	0	3
Sago pondweed	100	22	96	31	76	0	100	0	3
Star-grass	100	-4	73	6	65	0	50	0	1

(Continued)

Table 15 (Concluded)

Treatment	Removal of Nitrobodies								Number of Nitrobodies with >80% Removal
	TNT	RDX	TNB	TADNT	TDANT	TDNT	TDNB	NB	
Initial Concentration	2,123	2,934	294	78	78	<0.1	18	<0.1	
Fertilized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1} (Continued)									
Spikerush	100	2	70	-32	13	0	28	0	1
Stonewort	100	3	90	94	55	0	100	0	4
Fertilized Controls									
Groundwater	68	-5	19	-100	60	0	-11	0	1
Sediment	97	38	89	-58	37	0	100	0	3
Autocl.sediment	100	52	99	-6	32	0	100	0	3

8.3 in the incubation water, a range in which they usually are present (Channon, Mills, and Williams 1994).

Non-plant Contributions to Explosives Removal

TNT is known to degrade in biologically active systems relatively rapidly, and a certain proportion of plant-associated effects are expected to be attributable to microorganisms present on plant tissue. While it is assumed that these populations are significant in effect, this study did not quantify their contribution. In addition, photolysis of TNT and RDX occurs in the visible and UV wavelength ranges and can be substantial (Spanggord et al. 1980). The effect of UV radiation is expected to increase photolysis and result in more rapid explosive degradation in any treatment in an outdoors system. However, in the growth chambers, irradiance was relatively low, and the UV component was absent from the light spectrum. Photolysis was expected to be minimal.

Plant Health and Growth

Plant health was assessed by visual inspection twice during the incubation period, and relative growth rates were calculated from initial and final DW data. Inspection indicated that most plants had not recuperated from transplant shock by 7 days and only started to do so by 10 days. Almost all growth rates were found to be negative (Figure 7). Only parrot-feather at the higher density and with N-fertilizer showed net weight gain. This nutrient amendment was associated with less negative growth rates in all species except vallisneria, sago pondweed, and stonewort. The lack of growth and limited response to N may indicate that (a) the time required for recovery was too long for plants to take advantage of N amendment, (b) another major nutrient was more limiting than N, (c) growth potential of the plants was low, late in the growing season (October), or (d) initial explosives levels were inhibitory to the plants. The latter possibility is substantiated by

Table 16
Concentrations, $\mu g\ L^{-1}$, of RDX and TNT, TNT Metabolites, and TNT Photolysis Products in Initial Groundwater and Following 10-Day Incubation with Plants or Controls, Mean Values (N = 3)

Treatment	RDX	TNT	TNT Metabolites							TNT Photolysis Products			
			2ADNT	4ADNT	2,6DANT	2,4DANT	2,6DNT	2NT	3NT	TNB	1,4DNB	1,3DNB	NB
Initial													
Groundwater	2934	2123	42	36	72	6	-	-	-	294	-	18	-
After 10-Day Incubation													
Lower density - 9 g FW L^{-1}													
Parrot-feather	2257	16	12	101	23	-	-	1	-	1	-	-	-
Milfoil	2878	-	-	-	21	-	-	-	-	23	-	-	-
Egeria	2638	34	21	109	23	38	6	-	-	72	-	-	-
Elodea	2836	-	-	2	22	-	-	-	-	29	4	-	-
Vallisneria	3082	-	36	241	23	43	-	-	-	86	-	-	-
Curlyleaf p'weed	2789	-	16	77	-	-	-	-	-	-	-	-	-
Sago pondweed	2612	-	-	40	-	4	-	-	-	17	-	-	-
Star-grass	2966	-	13	82	20	4	-	-	-	78	-	-	-
Spikerush	2832	-	37	155	-	33	-	-	-	88	13	-	-
Stonewort	2740	-	-	-	24	5	-	-	6	61	-	-	-
Controls													
Groundwater	2840	471	91	93	-	2	-	-	-	169	-	10	7
Sediment	2759	450	130	162	25	4	-	-	-	135	23	9	-
Autocl.sediment	2600	25	103	312	-	39	-	-	-	59	-	3	-

Note: - = <0.1 $\mu g\ L^{-1}$.

findings that TNT levels of 1 mg L^{-1} depress growth of duckweed (*Lemna* spp.) (Schott and Worthley 1974).

The likelihood of growth limitation by nutrients was assessed from pH and macronutrient concentrations in the water at the end of the incubation period (Table 17). Alkalinity was generally low (initial value 1.25 mM) and in the range to produce carbon limitation for submersed plants (Van, Haller, and Bowes 1976). Final pH ranged from 5.3 to 8.4. The lower levels may have inhibited carbon availability in species preferring bicarbonate-carbon for photosynthesis: Eurasian watermilfoil, curlyleaf pondweed, sago pondweed and vallisneria (Spence and Maberly 1985). Without water mixing, carbon transport may have been limited to diffusion alone and would have affected all plant species, irrespective of CO_2 or bicarbonate carbon source use (Walker 1985). Only parrot-feather would have had access to CO_2 in air, where diffusion is far higher than in water. Lack of water mixing may be inferred from the low O_2 values determined at the end of the incubation period, approximately 1 to 8 mg L^{-1} (Figure 3).

Low light is not expected to have limited growth, as light levels in the growth chambers were close to the range in which photosynthesis of submersed plants is saturated (600 to 800 $\mu E\ m^{-1}\ s^{-1}$) (Van, Haller, and Bowes 1976).

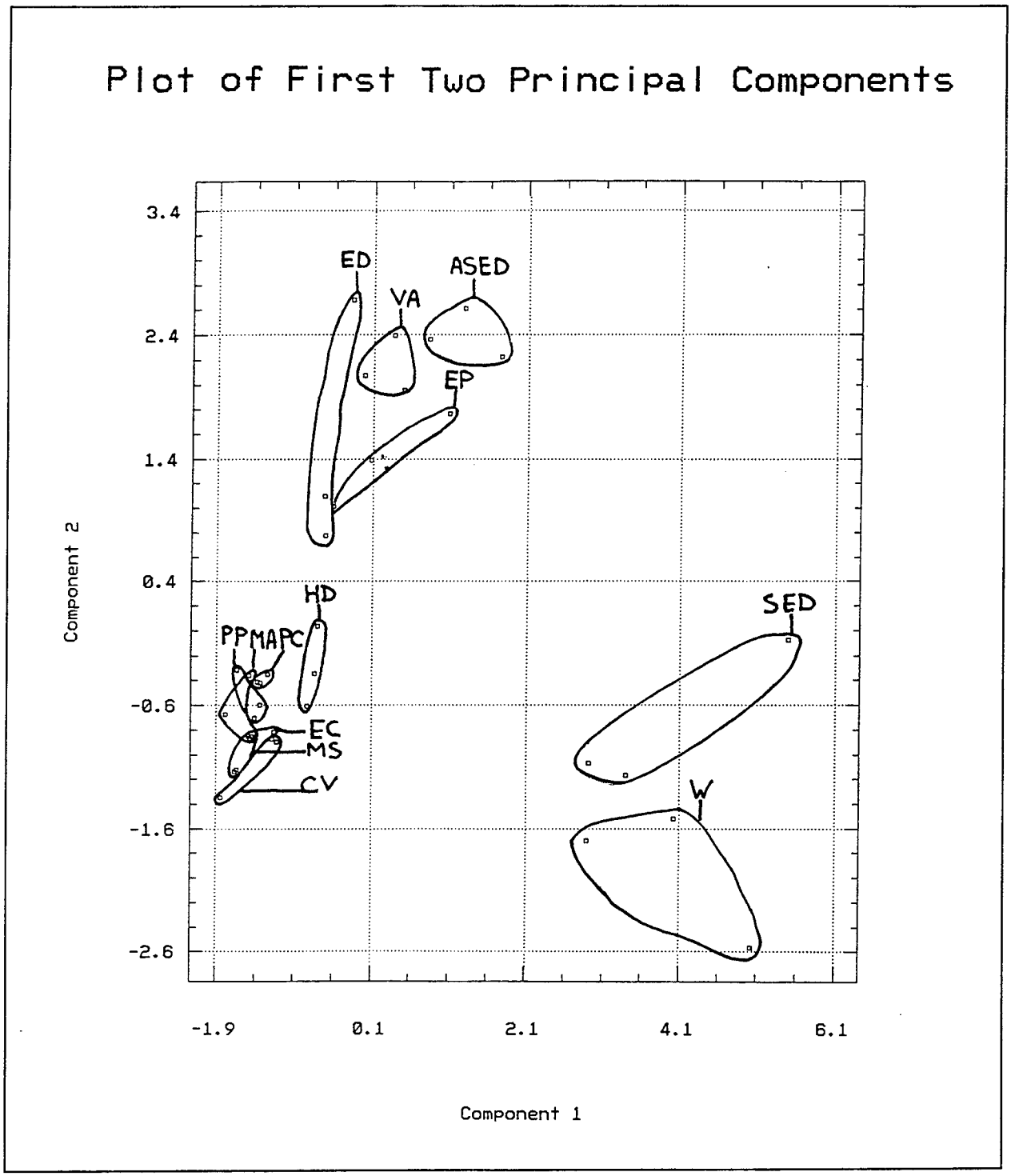

Figure 5. Scatterplot on the first two axes following principal component analysis of RDX, TNT, TNT metabolites, and TNT photolysis product concentrations in groundwater following 10-day incubation with plants or controls. Abbreviations of plant names are defined in Appendix D.

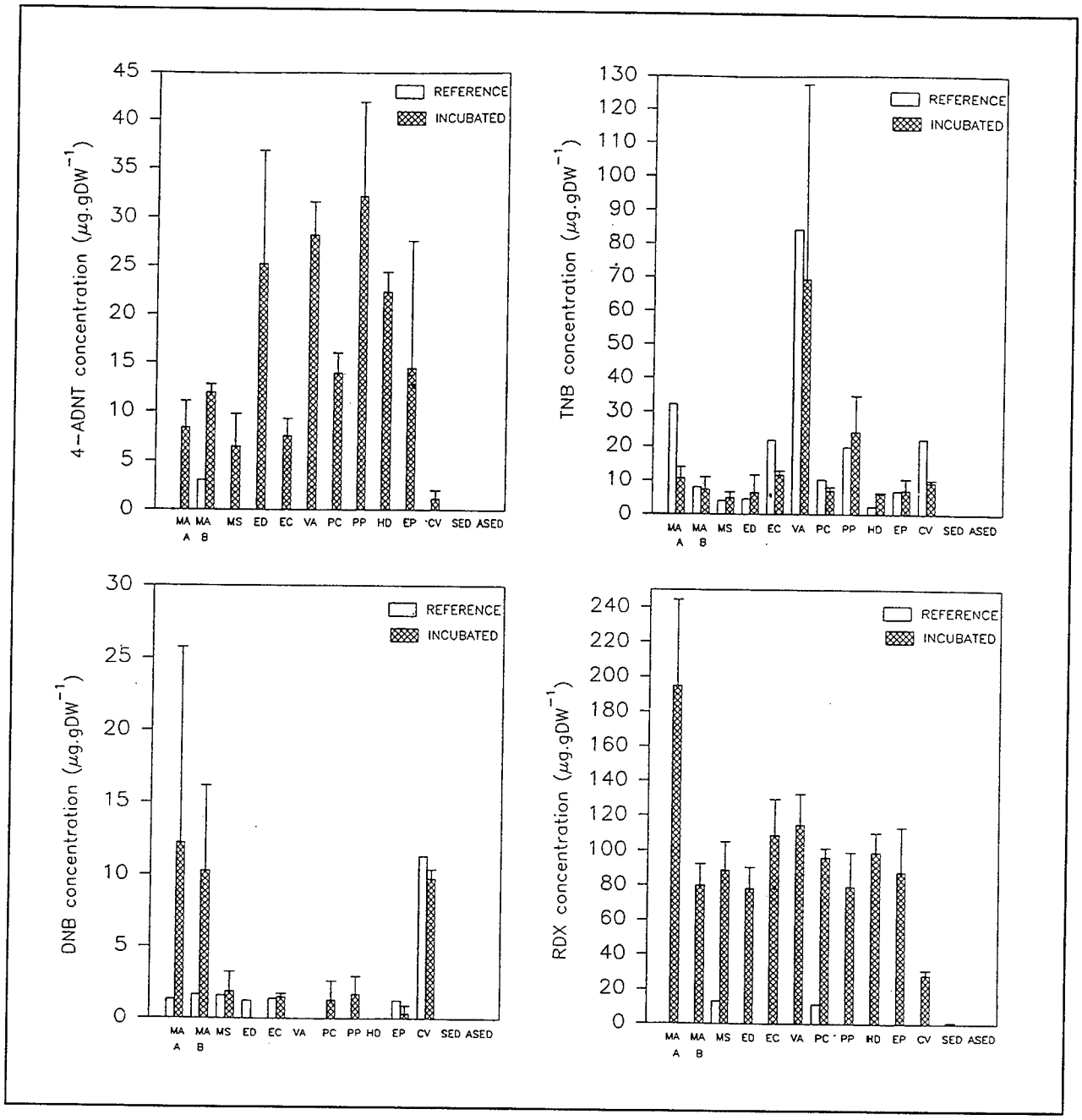

Figure 6. Concentrations of reduction (4ADNT) and photolysis (TNB and DNB) products of TNT and of RDX in plants and sediments exposed to explosives-contaminated groundwater following 10-day incubation and in nonexposed reference plants. Exposed: mean values and standard deviations (N = 3); reference: single values. Abbreviations of plant names are defined in Appendix D.

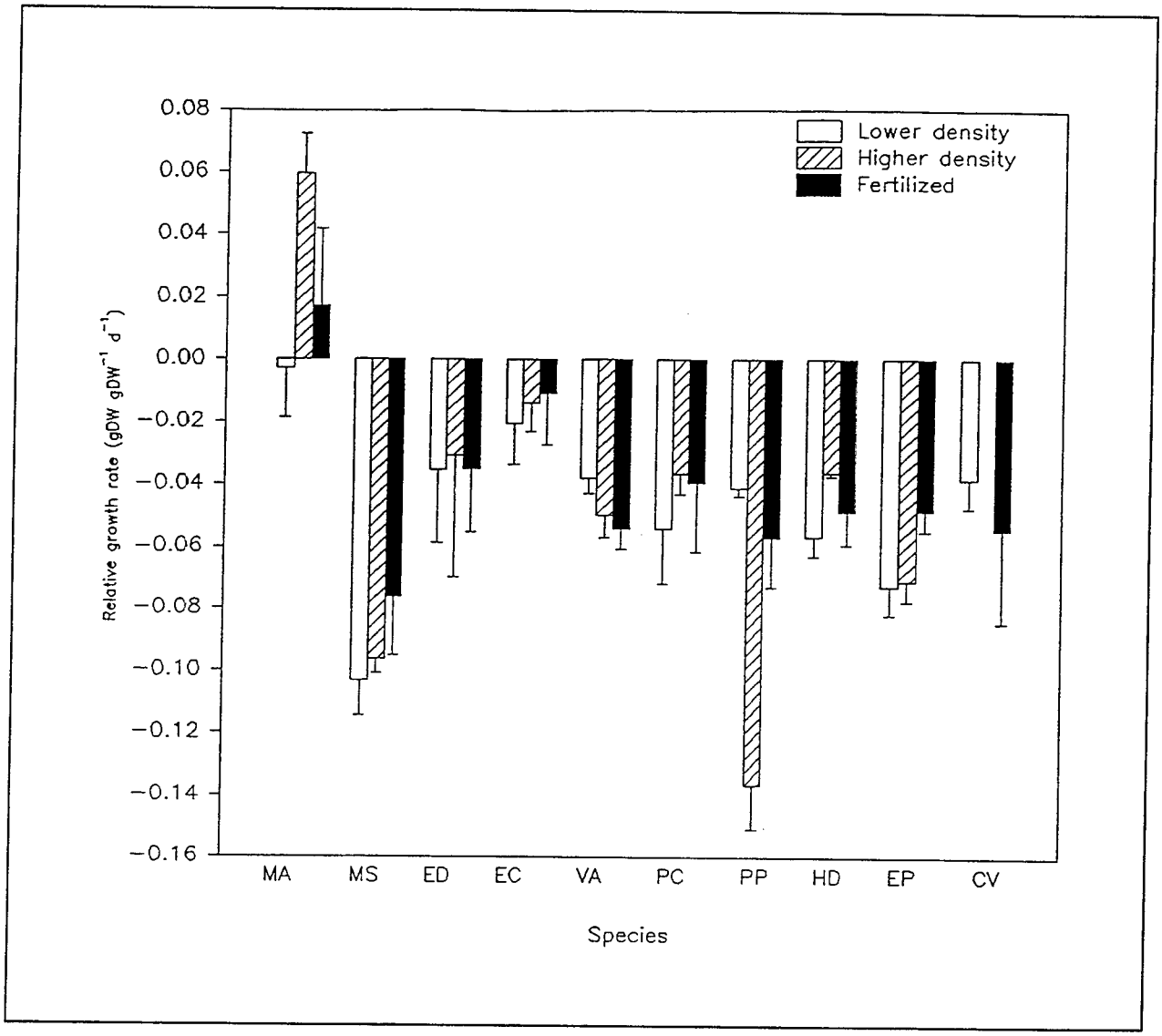

Figure 7. Relative growth rates of aquatic plant species cultivated for 10 days in explosives-contaminated groundwater. Mean values and standard deviations (N = 3). Abbreviations of plant names are defined in Appendix D.

Table 17
Chemical Characteristics of Initial Groundwater and Following 10-Day Incubation, Mean Values and Standard Deviations (N = 3)

Pseudospecies	pH	Alkalinity mg L^{-1} Ca CO_3	NO_3-N mg L^{-1}	NH_4-N mg L^{-1}	SRP mg L^{-1}	SO_4 mg L^{-1}	Ca mg L^{-1}
Initial							
Groundwater	8.3 ± 0.1	15 ± 3	5.8 ± 1.7	0.08 ± 0.08	0.179 ± 0.034	1.53 ± 0.16	5.9 ± 1.3
After 10-Day Incubation							
Lower density - 9 g FW L^{-1}							
Parrot-feather	7.0 ± 0.2	20 ± 4	5.3 ± 2.1	0.10 ± 0.05	0.147 ± 0.104	1.41 ± 0.10	5.6 ± 1.3
Milfoil	7.1 ± 0.2	43 ± 10	3.5 ± 0.2	0.28 ± 0.05	0.868 ± 0.224	1.36 ± 0.07	9.7 ± 3.2
Egeria	8.4 ± 0.5	37 ± 6	8.8 ± 1.2	0.08 ± 0.05	0.103 ± 0.082	1.34 ± 0.14	10.3 ± 3.8
Elodea	7.0 ± 0.1	19 ± 6	5.6 ± 1.4	0.34 ± 0.20	0.244 ± 0.120	1.33 ± 0.10	4.2 ± 0.1
Vallisneria	6.5 ± 0.5	19 ± 15	5.3 ± 3.1	0.13 ± 0.03	0.215 ± 0.022	1.67 ± 0.29	9.4 ± 4.0
Curlyleaf pondweed	6.4 ± 0.1	14 ± 3	6.1 ± 0.7	0.20 ± 0.04	0.297 ± 0.082	1.36 ± 0.16	4.1 ± 0.5
Sago pondweed	6.9 ± 0.1	40 ± 4	1.3 ± 0.4	0.10 ± 0.01	1.226 ± 0.174	6.62 ± 1.23	4.0 ± 0.4
Star-grass	6.1 ± 0.7	16 ± 14	8.3 ± 0.5	3.40 ± 0.63	1.273 ± 0.146	1.59 ± 0.33	3.4 ± 0.2
Spikerush	5.7 ± 0.4	5 ± 3	8.6 ± 0.3	0.49 ± 0.19	0. 860 ± 0.057	3.20 ± 1.55	2.6 ± 2.1
Stonewort	7.8 ± 0.0	108 ± 24	6.9 ± 0.3	0.18 ± 0.02	0.071 ± 0.022	2.46 ± 0.61	10.2 ± 2.2
Higher density - 18 g FW L^{-1}							
Parrot-feather	6.8 ± 0.2	40 ± 4	0.4 ± 0.4	0.19 ± 0.02	0.131 ± 0.018	1.61 ± 0.33	6.1 ± 2.3
Milfoil	7.0 ± 0.2	89 ± 18	0.0 ± 0.0	1.94 ± 1.78	2.400 ± 1.542	1.26 ± 0.21	13.2 ± 4.1
Egeria	7.9 ± 0.3	47 ± 2	9.4 ± 0.2	0.07 ± 0.01	0.071 ± 0.025	1.27 ± 0.06	12.0 ± 5.0
Elodea	6.9 ± 0.0	43 ± 6	1.5 ± 1.4	0.44 ± 0.19	0.474 ± 0.114	1.33 ± 0.08	5.0 ± 0.5
Vallisneria	7.0 ± 0.2	29 ± 9	5.2 ± 1.8	0.20 ± 0.01	0.242 ± 0.093	2.72 ± 0.74	9.0 ± 2.5
Curlyleaf pondweed	6.7 ± 0.2	48 ± 4	0.5 ± 0.4	0.45 ± 0.24	1.273 ± 0.332	2.05 ± 0.84	5.1 ± 1.0
Sago pondweed	7.2 ± 0.1	141 ± 18	1.2 ± 1.0	10.70 ± 3.22	3.336 ± 0.812	4.39 ± 0.34	6.1 ± 1.8
Star-grass	6.7 ± 0.3	28 ± 13	4.2 ± 2.1	6.26 ± 2.67	1.566 ± 0.531	2.15 ± 0.75	0.7 ± 0.3

(Continued)

Table 17 (Concluded)

Pseudospecies	pH	Alkalinity mg L^{-1} Ca CO_3	NO_3-N mg L^{-1}	NH_4-N mg L^{-1}	SRP mg L^{-1}	SO_4 mg L^{-1}	Ca mg L^{-1}
Higher density - 18 g FW L^{-1} (Continued)							
Spikerush	6.5 ± 0.3	14 ± 4	9.4 ± 7.9	0.27 ± 0.02	2.863 ± 2.182	5.19 ± 0.42	0.3 ± 0.0
Controls							
Groundwater	7.0 ± 0.3	10 ± 4	8.4 ± 0.2	0.04 ± 0.01	0.267 ± 0.026	1.63 ± 0.07	5.0 ± 0.2
Sediment	7.8 ± 0.2	52 ± 2	6.0 ± 0.3	0.07 ± 0.01	0.105 ± 0.003	4.77 ± 3.13	7.8 ± 0.9
Autoclaved sediment	7.5 ± 0.2	67 ± 5	2.4 ± 0.3	0.17 ± 0.08	0.222 ± 0.049	1.29 ± 0.16	11.8 ± 2.9
Fertlized - 50 mg NO_3-N L^{-1}; 9 g FW L^{-1}							
Parrot-feather	6.8 ± 0.2	33 ± 10	42.9 ± 1.2	0.10 ± 0.06	0.088 ± 0.013	1.42 ± 0.16	7.8 ± 1.1
Milfoil	7.3 ± 0.0	60 ± 11	31.7 ± 7.9	0.25 ± 0.04	0.800 ± 0.181	1.36 ± 0.12	13.2 ± 4.7
Egeria	8.4 ± 0.6	42 ± 1	47.2 ± 0.3	0.08 ± 0.01	0.073 ± 0.003	1.40 ± 0.12	12.6 ± 3.4
Elodea	6.9 ± 0.1	38 ± 20	30.5 ± 5.6	1.10 ± 0.46	0.188 ± 0.063	1.25 ± 0.04	6.0 ± 0.4
Vallisneria	6.5 ± 0.3	16 ± 6	45.1 ± 1.5	0.18 ± 0.04	0.275 ± 0.093	1.75 ± 0.19	9.3 ± 1.1
Curlyleaf pondweed	6.7 ± 0.1	19 ± 2	34.7 ± 7.5	0.29 ± 0.00	0.272 ± 0.102	1.35 ± 0.13	5.2 ± 2.0
Sago pondweed	6.9 ± 0.0	34 ± 1	30.0 ± 6.7	0.09 ± 0.00	0.743 ± 0.200	5.75 ± 0.32	5.6 ± 2.6
Star-grass	5.3 ± 0.6	4 ±3	39.7 ± 10.1	2.67 ± 0.52	0.843 ± 0.210	1.44 ± 0.14	4.6 ± 0.8
Spikerush	6.4 ± 0.1	20 ± 11	43.5 ± 7.6	0.19 ± 0.05	0.818 ± 0.165	3.24 ± 1.36	3.7 ± 2.2
Stonewort	7.9 ± 0.3	91 ± 15	32.4 ± 7.4	0.15 ± 0.03	0.039 ± 0.018	5.56 ± 2.33	15.7 ± 7.0
Fertilized Controls							
Groundwater	6.7 ± 0.1	6 ± 0	37.6 ± 12.8	0.07 ± 0.03	0.133 ± 0.118	1.36 ± 0.15	4.1 ± 1.0
Sediment	7.9 ± 0.2	70 ± 10	23.4 ± 8.2	0.10 ± 0.01	0.015 ± 0.019	1.86 ±0.35	6.0 ± 2.8
Autoclaved sediment	7.3 ± 0.1	69 ± 7	27.7 ± 4.6	0.09 ± 0.01	0.027 ± 0.021	1.58 ± 0.23	11.2 ± 2.9

4 Conclusions

Removal Processes and Kinetics

All ten plant species enhanced the rate of removal of TNT from MAAP groundwater, and this plant-associated effect increased with increasing biomass. Estimated residence times required for cleanup to 2 μg TNT L^{-1} were reduced from 56 days in water alone to 6 to 8 days when plants of almost all species were included in the incubation at 9 g FW L^{-1}. At twice this FW density, residence time was reduced by 2 days. These cleanup periods with plants compare to residence times of 55 days in unautoclaved sediment incubated with water alone, and 16 days when sediment was autoclaved. Nitrogen fertilization did not enhance removal activity in most plant species, although this may have been due to an overall lack of growth. However, nitrogen significantly enhanced removal associated with sediment, decreasing residence times to 19 days when sediment was not autoclaved, and to 7 days when it was.

Regression analysis suggests that plant-associated TNT removal can be ascribed to two processes, adsorption to tissue early in the incubation period, and metabolism at a rate described by the intercept and the slope, or rate, of regression. While the biological processes associated with TNT removal occurring in plant incubations may be a combination of adsorption to plant tissue, uptake and metabolism by plants, and metabolism by microorganisms, only degradative products indicative of reductive pathways were found.

PCA also showed that the presence of plants produces a TNT remediation kinetic that is different from that occurring in groundwater alone or with unautoclaved sediment. However, the similar behavior of autoclaved sediment to that of several plant species suggests that these types of treatments share common removal processes.

The extremely variable plant effects on RDX removal may be related to the long residence time required to lower levels of this compound ($t_{½} \geq 12$ days has been reported (Spanggord et al. 1980)) and to problems with analysis. Higher plant density enhanced the process. RDX degradation products were not screened.

Species Recommendations

Using calculations of retention times for TNT and results from multiple-range analyses, and with some consideration of RDX removal, the submersed species that were recommended for Phase II of the demonstration project for explosives removal from MAAP groundwater in constructed aquatic plant lagoons were elodea, sago pondweed, waterstargrass, and curlyleaf pondweed. The biomass normalized rate of removal, *K/DW*, suggests that vallisneria was also a suitable candidate.

Future Work

Evaluation of the efficacy of phytoremediation will require investigations that can separate the effects of plant adsorption and metabolism under flow-through conditions, compare them to the effects of microorganisms, and elucidate the role of photolysis in explosives degradation. Clarifying these processes in explosives removal will require techniques such as the use of radiotracers to determine mass balances, sterile plant cultures, and photochemistry.

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Appendix A
Standard Protocol for Species Screening

Phytoremediation of Explosives-Contaminated Groundwater Using Constructed Wetlands

Standard Protocol for Species Screening

October 1995

This document outlines a set of standard practices for screening aquatic and wetland plants in batch systems for relative ability to reduce explosives levels in contaminated groundwater. These evaluations of submersed and emergent species will be carried out by the U.S. Army Engineer Waterways Experiment Station (WES) and Tennessee Valley Authority (TVA), respectively, as an initial part of the joint agency Environmental Security Technology Certification Program (ESTCP) project "Phytoremediation of Explosives-Contaminated Groundwater Using Constructed Wetlands".

While conceding innate differences between submersed and emergent forms and activities, this protocol allows the two studies to be analogous for as many elements as possible and facilitates comparison of results.

Plant Factors

In these evaluations, 10 submersed and 10 emergent plant species will be exposed to site groundwater from the Milan Army Ammunition Plant (MAAP) for a period of 10 days. Choice of species for evaluation has been based on a range of characters, including perenniality, high year-round biomass, extensive root/ rhizome systems, survival under anaerobiosis, and information on presence of nitroreductase enzyme activity.

Density: Ratio of plant biomass to contaminated water

In submersed species, most groundwater transformation is expected to occur in foliage, which represents the majority of the plant's biomass. In emergents, transformation is assumed to be proportional to the amount of root and crown biomass in contact with water; the ratio of these parts to the whole plant will differ by species.

Submersed. Field studies of highly productive aquatic plants cite densities of from 300 g dry weight m^{-2} for Eurasian watermilfoil (Grace and Wetzel 1978) to 9 kg fresh weight (fr wt) m^{-2} for hydrilla (Bowes, Holaday, and Haller 1979). Field capacity thus approximates 3 to 9 g fr wt L^{-1}. Proposed test densities of 9 and 18 g fr wt/L are based on the possibility of nutrient and management inputs. Density is by final total volume, i.e., a 1-L volume will contain 9 or 18 g plant material.

Emergents. Measured as a standing crop, densities of 45 and 90 g fr wt L^{-1} are proposed on the basis of approximately 20 percent of total biomass being below the water surface. This maintains a proportional relationship to the densities used for submersed species.

Root:shoot and emersed:submersed ratios. Data will be recorded for emergent species on ratio of root to shoot and proportion of emersed biomass. Root:shoot ratios will be determined in submersed species where rooting occurs.

Propagation and acclimation

Submersed. For most species, apical shoots from actively growing plants will be used as propagules for the evaluation. Plants with few stem nodes (e.g., pondweeds) or only basal meristems (e.g., vallisneria) will be evaluated as whole plants or trimmed crowns with attached root/rhizome.

Emergents. Whole plants with attached root/rhizome will be used.

Acclimation. One to two weeks hydroponic growth in 0.25x Hoagland's nutrient culture medium (Hoagland and Arnon 1938). This will allow plants to overcome transplant shock in a clean environment and limit algal or bacterial associations. The Hoagland's formulation will be the one currently used by the TVA (Table A1).

Sources of plants. Plants will be obtained from commercial nurseries, governmental agencies, or local field collection.

Initiate experiments. Experiments will begin within a week of October 1, 1995.

Culture Conditions During Treatment

Lighting

Submersed. The lighting will be artificial, supplied by high-pressure sodium and metal halide lamps. Average photosynthetic photon flux density (PPFD) at water surface expected to be 500 to 600 $\mu E\ m^{-2}\ sec^{-1}$; recorded at intervals during experiment. No ultra-violet (UV) radiation present. Sides of experimental units will be covered to simulate underwater light conditions.

Emergents. The lighting will be natural lighting incident to greenhouse, recorded by sensor during experiment. No UV. Sides and surfaces of experimental units will be covered to simulate root zone conditions.

Daylength. For emergents, the daylength will be the natural daylength of Muscle Shoals, AL, during this time period; for submersed, a similar light regime, approximately 12 hr light, 12 hr dark (12L:12D).

Temperature

Submersed. The temperature will be set to 25 °C with a range of ±2 °C, recorded during evaluation.

Emergents. The temperature will be that naturally occurring under greenhouse conditions, recorded during course of experiment.

Culture containers and substrate

Experimental units and plant support systems that contact contaminated water will be constructed of glass, stainless steel, teflon (TFE), or other inert materials such as polypropylene plastic. Units will be acclimated to groundwater by filling for 24 hr before test begins; this water will then be discarded.

Submersed. The containers will be glass aquaria constructed with silicone sealant; 37.5 cm tall x 15 cm square. The test volume will be 3.375 L water, resulting in 30.4 g fr wt plant material per aquaria at the lower density (9 $g\ L^{-1}$), and 60.8 g at the higher (18 $g\ L^{-1}$).

Emergents. The containers will be glass vessels, approximately 3.8 L. Plants will be anchored in plastic pots that have been shown to have nonsignificant explosives adsorption over a 6-day period.

Aeration

In order to duplicate the low oxygen exchange conditions expected in the field, no aeration will be provided. Prior to each sampling event, liquid contents of the experimental unit will be stirred three times to normalize concentration.

Growth medium/solution

The growth medium will be groundwater, Well MI 146.

Nutrient amendment

This will be done only to support the test factor of fertilization.

Experimental Design and Test Factors

Experimental design

In order to limit the number of experimental units required, two interlocking synchronous experiments will screen species for explosives removal at differing planting densities and with or without fertilizer amendment, with three replications of each treatment combination. One experiment will test the effects of species and planting density, and their interaction, on the concentration of explosives, using 10 test species (including parrot-feather) at low and high density. A statistically separate experiment will test the effects of species and nitrogen fertilization, and their interaction, using the same 10 species, with nitrogen amendment at the lower planting density. Sediment effects will be examined separately by WES.

Submersed. A randomized complete block (RCB) design will allow blocking by light level. Each block will consist of 32 units, comprising 10 species in three combinations of factors (low density and no fertilization; high density and no fertilization; low density with fertilization) and two water-only controls at two factor combinations (no fertilization; fertilization) for a total of 96 experimental units.

Emergents. A completely randomized design (CRD) will be used, with each of 10 species in 3 combinations of factors, and water-only controls, as above, for a total of 96 experimental units.

Statistical analysis. This will be carried out separately for each evaluation, using α-levels ≤ 0.05. Comparisons will be made between submersed and emergent species using means.

Sediment effects. The effect of sediment alone on explosives removal will be tested at WES under conditions similar to the submersed species evaluations. Autoclaved and unautoclaved aliquots of a MAAP sediment will be placed in contact

with the test groundwater, and samples of overlaying water will be taken. These tests will involve seven replications of each sediment condition.

Groundwater

MAAP groundwater from Well MI 146 will be used. Water from the same sample batch will be dispersed by TVA to WES for common use.

Initial characterization of the groundwater batch to determine concentrations of explosives and their degradation products, and Hg, Pb, Al, Fe, Mg, Mn, K, Na, Zn, Ca, NO_3-N, NO_2-N, alkalinity, NH_4-N, ortho-P, bicarbonate and pH, will be carried out by TVA.

Plant density

Submersed density will be 9 g and 18 g fr wt L^{-1}, and emergent density will be 45 g and 90 g standing crop L^{-1}.

Fertilization

A test of nitrogen (N) amendment alone is based on the assumption that (a) the largest response will result from N fertilization; (b) response to a single amendment can be more narrowly defined; and (c) macronutrients other than N will be available from groundwater. The lowest plant density level will be amended with NO_3-N, using KNO_3 at levels 0 and 50 mg L^{-1} NO_3-N, giving an actual level of approximately 10 and 60 mg L^{-1} NO_3-N (based on expected groundwater levels of approximately 10 mg L^{-1})

Controls

Effect of species, experimental unit, and fertilizer amendment will be controlled in these experiments in the following ways:

a. *Plant species*. Parrot-feather (*Myriophyllum aquaticum* (Vell.) Verdc.) included as one of the species tested in both submersed and emergent evaluations. Supplied from a single source near Muscle Shoals, AL, by TVA.

b. *Abiotic/nonplant transformation*. The experimental unit with groundwater and any support or light-limiting structures used, without plants.

c. *Fertilizer*. Experimental units with standard volume of groundwater amended with 50 mg L^{-1} NO_3-N, without plants.

Plant growth and survival

Ability of plants to survive and grow in MAAP groundwater will be assessed by monitoring increase in biomass over the 10-day incubation period.

Sampling and Analytical

Water

Water samples will be collected into glass bottles w/TFE-lined caps to prevent adsorption of explosives and ortho-phosphate.

Explosives analysis. To assess removal of explosives and their degradation products, 100-ml samples will be collected from each experimental unit at five sampling times. Samples will be taken at 24 hr and 10 days in both the submersed and emergent experiments. Three other sampling times will be scheduled prior to 24 hr in submersed, and following 24 hr in the emergents.

Explosive samples will be analyzed for the following:

a. Nitrobodies: TNT, RDX, 2,4-dinitrotoluene (2,4-DNT), nitrobenzene, trinitrobenzene

b. Explosives and TNT-metabolites: HMX, 2,4-DNT, 2,6-DNT, 2-Am-4,6-DNT, 4-Am-2,6-DNT, 2-Diam-NT's, TAT, mononitrotoluene

The HPLC analytical methods used are modifications of EPA Method 8330 (USEPA 1990), with the concentration step of the 100-ml water samples using solid phase extraction.

Nutrient analysis. 100-ml samples will be taken at the beginning and end of the experiment. Water pH will be determined immediately; NH_4-N, NO_3-N, ortho-P, and bicarbonate will be analyzed later.

Note: Water volume in the experimental units will decrease due to sampling. To deal with this and with evaporative loss, known amounts of deionized water may be added at 5 days to readjust to original volume. Records will be kept of all additions.

Plant material

Initial biomass will be taken as fresh weight, determined immediately before planting material is placed into site water.

At the end of the incubation all plant material will be harvested, and fresh weight of above- and below-water portions will be measured separately. Fresh

weight:dry weight ratios for each species will be determined from a portion of the material, after drying to a constant weight, approximately 24 - 48 hr at 70 °C. Remaining fresh material will be frozen (-20 °C) for later explosives analysis.

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Table A1
Hoagland's Solution Formulation, Quarter Strength as Formulated by TVA

Compound	MW	g L^{-1} in Stock Solution	ml Stock L^{-1} in Final	Element	Element Concentration ppm
NH_4NO_3	80	160	1.0	N	56.0
$CaCl_2.2H_2O$	147.1	117.7	0.5	Ca	16.0
KH_2PO_4	136	54.4	0.5	Cl	32.0
K_2SO_4	174	87.0	0.5	K	27.4
$MgSO_4.7H_2O$	246.5	247	0.5	Mg	12.2
NaCl	58.5	11.7	0.5	Na	8.3
				P	6.2
				S	24.0
Micronutrient Compound	**MW**	**mg L^{-1} in Stock Solution**	**ml L^{-1} Stock in Final**	**Element**	**Element Concentration ppm**
H_3BO_3	61.8	248	0.25	B	10.8
$CoCl_2.6H_2O$	238	952	0.25	Co	58.9
$MnSO_4.H_2O$	169	676	0.25	Mn	54.9
$Na_2MoO_4.2H_2O$	242	194	0.25	Mo	19.2
$ZnSO_4.7H_2O$	288	230	0.25	Zn	13.1
$CuSO_4.5H_2O$	250	50	0.5	Cu	6.35
Fe EDDHA 6% Fe	--	10,000	5.0	Fe	3000

Appendix B
Site Visit to Milan Army Ammunition Plant, Milan, TN, 13 September 1995

The Milan Army Ammunition Plant (MAAP) site visit was conducted to allow participants in the joint agency ESTCP project "Phytoremediation of Explosives-Contaminated Groundwater Using Constructed Wetlands" to become familiar with local plant communities of aquatic and wetland species, and to review suitable submersed and emergent candidates for laboratory evaluations and for deployment in a field treatability study.

Participants from cooperating agencies were Drs. Les Behrends, Paul Pier, and David Webb of the TVA, Environmental Research Center; Dr. Mike Saunders of the Georgia Institute of Technology, accompanied by Mr. Paul Palazolo of the Ground Water Institute of the University of Memphis; and Dr. Susan Sprecher of the USACE Waterways Experiment Station. Mr. Mike Robinson, of Lockheed Martin Ordnance Systems, facilitated the visit with the help of Mr. Pat Brew, MAAP.

Participants met at the Administration Building at 0730, where they were welcomed by Robinson and Brew, and greeted by the Commander, MAAP. Robinson stayed with the group for the duration of the visit, and provided transportation.

The morning began with a brief discussion of the range of characters being used to choose plants for the factorial evaluations of transformation in the laboratory and for the field treatability study; these included perenniality, high year-round biomass, extensive root/rhizome systems, survival under anaerobiosis, and presence of nitroreductase enzyme activity. Selected species are expected to be screened for the presence of the nitroreductase enzyme. The status of these emergent and submersed species as weedy or noxious was discussed in regard to a draft report currently under discussion (only) by the Research Committee of the Tennessee Exotic Pest Plant Council. Those plants being considered for project evaluation and deployment are rated as follows, in decreasing order of threat:

a. *Submersed.*

(1) "Severe Threat": *Myriophyllum spicatum* (Eurasian watermilfoil)

(2) "Significant Threat": *Egeria densa* (egeria, Brazilian elodea); *Myriophyllum aquaticum* (parrotfeather)

(3) "Lesser Threat": *Potamogeton crispus* (curly pondweed)

b. *Emergent.*

(1) "Severe Threat": *Phalaris arundinacea* (canary grass); *Phragmites australis* (common or wild reed cane)

The potential deployment of these and other weedy or exotic species in the treatability and field studies continued to be discussed during the course of the day. Canary grass, reed, water hyacinth, Eurasian watermilfoil, and elodea have already proved successful in constructed wetlands and/or TNT transformation. Some contribute activity under anaerobic conditions (milfoil, canary grass), or under high temperatures (water hyacinth) that reduce physiological activity in other plants (parrot-feather, milfoil). Various control measures are available: for example, water hyacinth is naturally controlled by freezing temperatures; seed heads of canary grass and reed could be clipped before seeds are shed, or prevented from forming by application of plant growth regulators; reproductive fragments of milfoil or egeria can be contained in a catchment area and destroyed. While canary grass was not seen during the visit, Webb indicated that it is reported from west Tennessee. Robinson said that Steve Stevenson, MAAP, has a copy of the Natural Resources Plan for the facility, and it is possible that this contains a list of unwanted plant species. Sprecher will contact Stevenson about this.

At 0900 the group moved to the field, with the first stop at the K Line location. Wells K100, an old process well, and MI 146, a monitoring well approximately 50 yards away, were examined. There was discussion of the relative merits of the wells as sources of groundwater for the laboratory evaluations and the field treatability study. MI 146 provides 27 gal min^{-1} and has been characterized for contaminants and mineral content. Information on K100 is not yet available as the pump currently in place produces 1,000 gal min^{-1}, creating wastewater disposal problems. TVA currently has approximately 1,000 gal of MI 146 output, and agreed to provide WES with water so that both factorial species screening studies can be run with the same batch of water. The pH of incoming water is expected to be 5.5 to 6.0. The facilities available in the pump house over K100 were monitored; the K9 building had electricity and a floor drain.

The group then moved off the MAAP facility to a roadside landing with an inundated area of shallow, slowly flowing (lentic) water, along the floodplain of the Middle Fork of Forked Deer River, about 2 miles NW of Spring Creek along Hwy 15. The dominant emergent was *Nuphar luteum* (spatterdock, yellow waterlily, cow lily, bullhead lily) present in large stands. Other wetland species were *Scirpus cyperinus* (woolgrass), *Sparganium* sp. (burreed), *Peltandra virginica*

(green arum), *Typha latifolia* (cattail), *Polygonum hydropiperoides* and *P. punctatum* (water smartweeds), and *Saururus cernuus* (lizard tail). The large perennial underwater rhizomes (4 to 6 cm thick, starchy, edible) and numerous fibrous roots of spatterdock were examined. The submersed *Cabomba caroliniana* (cabomba, fanwort) was found in association with spatterdock, but otherwise submersed species were rare here and at the other wetland areas examined in the course of the day. This may be due to the presence of murky water with high turbidity during some portion of the growing season, preventing photosynthetic activity of submersed plants. It may also be a function of the high organic matter content of the sediments in these marsh areas; submersed species often have difficulty establishing in this type of substrate.

Luxuriant stands of parrot-feather were found growing in a roadside borrow ditch along the floodplain of the Middle Fork of Forked Deer River, about two miles SW of Cedar Grove along Hwy US 70. This species is obviously acclimated to the area, and appears to have become naturalized in spite of being an exotic. The emergent stalks stood 35 to 45 cm high, and the submersed stems were even longer, growing horizontally above a detrital mat of older stems. The stand appeared to be limited to water less than approximately 2 ft (60 cm) deep. Associated species were spatterdock, smartweeds, button bush (*Cephalanthus occidentalis*), *Lemna* spp. (duckweeds), *Ludwigia peploides* (water primrose), and woolgrass.

Following lunch at MAAP, Robinson took TVA and WES personnel to Trezevant Bottoms. This area, approximately 15 miles northeast of the installation, comprises a floodplain of the South Fork of the Obion River, ca. 6 miles NE of Trezevant along US 79. The extensive swamps/wetlands on both sides of the highway were dominated by emergent spatterdock in shallow areas, and a tall, thick-stemmed smartweed (*Polygonum densiflorum*) that extended from the banks out over open water. The amount of biomass produced by these two species was immense. Mats of detritus already resulting from this year's growth of smartweed were overgrown by the new shoots that readily form fibrous roots at leaf nodes. Lizard's tail with numerous seedheads, *Hibiscus* spp. (mallow), buttonbush, *Alnus* (alder), and intermediate-sized water smartweeds (*Polygonum hydropiperoides* and another *Polygonum* sp.) were common on the edge; extensive dense stands of woolgrass were associated with cattails. Smaller herbaceous species included green arum, *Hydrocotyle ranunculoides* (water pennywort, an exotic), duckweed, and *Azolla caroliniana* (azolla, mosquito fern). Webb noted that he collected the submersed *Ceratophyllum echinatum* (hornwort, coontail) at this site 20 years ago, although it was not seen on this trip.

During this time, Saunders and Palazolo verified access to the pumping facility in the K9 building, and checked water analysis results with laboratory personnel.

Back at MAAP, the reunited group had a general discussion of the field treatability study and the way in which various species will be incorporated into it. Saunders emphasized the need for plants to be deployed as established specimens in containers with suitable soil, to allow rapid acclimation and physiological activity during the autumn. He noted that milfoil does poorly without sediment. He also emphasized his need to know by October 1 (i.e., ASAP) what species are

recommended by the factorial studies, and where he will be able to get the necessary quantities of established plants. TVA said that it has stocks of emergents available for this. It was noted that the water in the treatability study lagoons will be at ambient (air) temperature rather than at in-ground temperature (≥ 55 °F). Species known to remain green during winter, and thus expected to have year-long physiological activity, include *Nasturtium officinale* (watercress; however, this is currently listed as a "severe threat" species by the Tennessee Exotic Pest Plant Council), *Juncus alpinus* (alpine rush; not native or naturalized), and *Equisetum* spp. (horsetails).

TVA noted that the rock filter (gravel beds, reciprocating with air lifts) part of the constructed wetland system would contain only emergent species; parrot-feather is expected to do well in this system where flow over root and submersed stem surfaces would support transformation. The lagoon system would contain mostly submersed species. The need for oxidization of anaerobic products coming out of anaerobic zones at the end of the transformation process was emphasized by TVA.

WES appreciates the support of the MAAP personnel and project participants on this visit.

Susan L. Sprecher, USCEWES-ES-P
Chemical Control and Physiological Processes

Appendix C
Analytical Specifications, Calibration Compounds, and Method References

HPLC Analysis of Explosives in Water

First, 100-ml samples were concentrated using solid phase extraction (SPE; Waters RDX cartridges, no. 47220; Jenkins et al. 1995).[1] In a few samples, plant debris was removed using Miracloth gauze. Subsequently, the explosives were eluted from the cartridges using acetonitrile. The samples were evaporated almost to dryness using N_2, redissolved in a 2-ml mixture of acetonitrile:water (50/50 v/v), and subsequently analyzed using High Pressure Liquid Chromatography (HPLC).

HPLC separations were performed on a Hewlett-Packard 1090 Series 2/M with ChemStation (Pascal Series) liquid chromatograph equipped with a diode array detector (Series 2), PV5 ternary solvent delivery system, thermostatically controlled column compartment, autosampler, auto-injector, and reverse phase analytical C18 column (5µ, 100 x 4.6 mm inner diameter) and ODS guard column (5 µ, 20 x 4.0 mm inner diameter). The column compartment was operated at 40 °C and the flow rate of the mobile phase was 1.5 ml min^{-1}. The composition of the mobile phase was 68 percent 20 Mm NH_4Cl and a 32 percent mixture of methanol and n-butanol (98:2, respectively).

The following compounds were used for the calibrations:

a. RDX (obtained from NEN Research, Boston, MA).

b. 1,3-Dinitrobenzene; 2,4-Dinitrotoluene; 2,6-Dinitrotoluene; 5-Nitro-1, 3-Dimethylbenzene (Aldrich Chemical Company, Milwaukee, WI).

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[1] References cited in this appendix are listed in the References at the end of the main text.

c. 1,3,5-Trinitrobenzene; 2,4,6-Triaminotoluene; 2,4,6-Trinitrotoluene; 2-Nitrotoluene; 3-Nitrotoluene; 4-Nitrotoluene; Nitrobenzene (Chem Service Chemicals, West Chester, PA).

d. 2,4-Diamino-6-Nitrotoluene; 2,6-Diamino-4-Nitrotoluene; 2-Amino-4, 6-Dinitrotoluene; 4-Amino-2,6-Dinitrotoluene; 4-Hydroxyamino-2,6-Dinitrotoluene, and the azoxy compounds: 4,4',6,6'-Tetranitro-2,2'-Azoxy-toluene and 2,2',6,6'-Tetranitro-4,4'-Azoxytoluene (Dr. R. Spanggord, SRI International).

Mean values on RDX and TNT of the initial water samples compare well. The SPE-RDX values are somewhat lower than the REF-values, possibly because some RDX remained on the solid phase cartridges after elution with acetonitrile.

A comparison was made of compounds and concentrations found by separate WES and TVA analyses of initial samples of the common batch of MAAP ground-water used for screening. The results are listed in Table C1.

Alkalinity, Macronutrients, and Calcium in Water

The pH was calibrated with known buffer solutions (American Public Health Association (APHA) 1992). Alkalinity was determined titrimetrically as $CaCO_3$ (APHA 1992, No 2320-B). NH4-N was measured using a selective ion electrode (Orion 95-12/Orion 940; APHA 1992, No 4500-NH3-G).

For the remaining analyses, the water samples were filtered over a 0.45 µm Gelman GN-6 filter. NO_3-N was measured using HPLC (Fa.Waters; APHA 1992, No 4500-NO3-C). SRP was measured spectrophotometrically using a Shimadzu 1201 UV/VIS Single Beam Spectrophotometer (APHA 1992, No 4500-PE). SO_4 was measured turbidimetrically (HACH Ratio turbidimeter; APHA 1992, No 4500-SO4-E). The concentration of total calcium (Ca) was determined by Atomic Absorption Spectrophotometry after acidification with 1:1 HCl to pH<2 (Varian Model SpectrAA-10; APHA 1992, No 3500-Ca).

The analytical precision and accuracy of determining macronutrients and Ca in water was checked by comparing the outcomes of determinations of 38 split water samples. The outcomes were usually similar.

Macronutrients, Bulk Density, and Organic Matter in Sediment

Total Kjeldahl nitrogen (N) and phosphorus (P) were determined in soil digests with sulfuric acid, potassium sulfate, and red mercuric oxide. N and P were measured colorimetrically using a Lachat Quikchem AE Automatic Flow Injection

Table C1
Comparison of Explosives Analysis in MAAP Groundwater Performed by WES and by TVA

Component	Concentration ($\mu g.L^{-1}$)	
	SPE	REF
HMX	NA	178 ± 5
2,6DNT	74 ± 3	NA
2,4DNT	7 ± 2	NA
RDX	3002 ± 82	3208 ± 77
TNB	308 ± 17	161 ± 6
1,4DNB	-	NA
1,3DNB	29 ± 14	NA
NB	-	NA
TNT	2197 ± 68	2187 ± 30
2ADNT	43 ± 1	158 ± 71
4ADNT	36 ± 1	45 ± 101
2,4DNT	-	NA
2,6DNT	-	NA
2NT	-	NA
4NT	-	NA
3NT	-	NA

Notes: Groundwater at the beginning of the experiment was analyzed in triplicate by WES and sixfold by TVA. In the WES analysis, 100-mL water samples were concentrated using solid phase extraction (SPE).
REF = Reference data determined by TVA, no concentration step.
NA = Not analyzed.
- = $<0.1\ \mu g\ L^{-1}$.

Ion Analyzer (QuikChem Methods No 10-107-06-2-D, 1992, and No 13-115-01-1-B, 1992). Exchangeable ammonium was extracted from the soil with 1 M NaCl and filtered; the filtrate was analyzed colorimetrically for ammonia via the salicylate method using a Lachat System (QuikChem Method No 12-107-06-2-A, 1988). Available P was extracted using a dilute HCl acid fluoride modified Bray extraction procedure and was analyzed colorimetrically via the ascorbic acid method using a Lachat System (QuikChem Method No 12-115-01-1-A, 1988).

Bulk density and moisture content were determined gravimetrically by drying a known quantity of fresh weight to constant dry weight at 105 °C (Allen et al. 1974). Concentrations of organic matter were determined by loss on ignition at 550 °C.

Appendix D
Abbreviations

Chemical Abbreviations

2ADNT	2-amino-4,6-dinitrotoluene
4ADNT	4-amino-2,6-dinitrotoluene
2,4DANT	2,4-diamino-6-nitrotoluene
2,6DANT	2,6-diamino-4-nitrotoluene
DNB	dinitrobenzene
1,3DNB	1,3-dinitrobenzene
1,4DNB	1,4-dinitrobenzene
DNT	dinitrotoluene
2,4DNT	2,4-dinitrotoluene
2,6DNT	2,6-dinitrotoluene
NB	nitrobenzene
2NT	2-nitrotoluene
3NT	3-nitrotoluene
4NT	4-nitrotoluene
NT	nitrotoluene
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
TADNTs	total monoamino-dinitrotoluenes (= 2ADNT, 4ADNT)
TDANTs	total diamino-nitrotoluenes (= 2,4DANT, 2,6DANT)
TDNBs	total dinitrobenzenes (=1,4DNB, 1,3DNB)
TNB	trinitrobenzene
TNT	2,4,6-trinitrotoluene

Plant and Treatment Abbreviations

ASED	Autoclaved sediment
CV	*Chara vulgaris* L., stonewort, muskgrass
DW	Dry weight, g
D1	Plant density of 9 g FW L^{-1}
D2	Plant density of 18 g FW L^{-1}

EC	*Elodea canadensis* Rich. in Michx., elodea, waterweed
EP	*Eleocharis parvula* (R. & S.) Link, dwarf spikerush
ED	*Egeria densa* Planch., egeria, Brazilian elodea
F1	Plant density of 9 g FW L^{-1} with no nitrogen amendment
F2	Plant density of 9 g FW L^{-1} amended with 50 mg NO_3-N L^{-1}
FW	Fresh weight, g
HD	*Heteranthera dubia* (Jacq.) MacM., water star-grass
MA	*Myriophyllum aquaticum* (Vell.) Verdc., parrot-feather
MS	*Myriophyllum spicatum* L., Eurasian watermilfoil
PC	*Potamogeton crispus* L., curlyleaf pondweed
PP	*Potamogeton pectinatus* L., sago pondweed
SED	Sediment
VA	*Vallisneria americana* Michx., vallisneria, wildcelery, tapegrass
W	Groundwater

Other

ANOVA	Analysis of variance
DoD	Department of Defense
HPLC	High-performance liquid chromatography
HSD	Honest significant difference
K	Removal rate constant
MAAP	Milan Army Ammunition Plant
PCA	Principal components analysis
SPE	Solid-phase extraction
SRP	Soluble reactive phosphorus
TVA	Tennessee Valley Authority
UV	Ultraviolet
WES	U.S. Army Engineer Waterways Experiment Station

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13. ABSTRACT *(Maximum 200 words)*

As an alternative to other groundwater extraction and surface treatment techniques, phytoremediation systems are currently being evaluated by civilian and military administrators for their ability to enhance removal of potentially toxic or mutagenic munitions matériel such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and their degradation products. To guide selection of aquatic plants for use in demonstration phytoremediation lagoons at the Milan Army Ammunition Plant (MAAP), Milan, TN, this study evaluated the relative ability of ten species to decrease levels of TNT and RDX explosives and related nitrobodies in contaminated MAAP groundwater.

The submersed macrophytes Eurasian watermilfoil, egeria, elodea, vallisneria, curlyleaf pondweed, sago pondweed, water star-grass, dwarf spikerush, and stonewort (an alga) were evaluated in laboratory-scale static hydroponic systems at two levels of plant density and nutrient amendment. These evaluations were carried out in conjunction with an examination of emergent species by the Tennessee Valley Authority (TVA), and the emergent parrot-feather was included in both studies for comparison. Plants were incubated in groundwater for 10 days, and water was sampled for explosives and metabolites via high-performance liquid chromatography (HPLC) analysis at 0, 1, 4, 12, 24, and 240 hr. Plant density was evaluated at 9 g fresh weight L^{-1} groundwater (approximate field capacity) and 18 g L^{-1}; a nitrogen amendment of 50 mg NO_3-N L^{-1} was applied as KNO_3 to plants at 9 g fresh weight L^{-1}. Plant tissue was analyzed by HPLC at the end of incubation.

(Continued)

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While little growth occurred during incubation, the presence of plants accelerated the rate of TNT removal over that in controls without plants. Incubations with elodea, sago pondweed, curlyleaf pondweed, stonewort, Eurasian wtermilfoil, water star-grass, and parrot-feather were significantly more effective than those with groundwater alone. The addition of fertilization or doubling of biomass did not enhance plant removal activity, and fertilization did not affect nonplant controls. Extrapolation of an exponential regression model (first-order kinetics) was used to compare rate of TNT removal and to estimate hydraulic retention time required to reach the potable water level of 2 μg TNT L^{-1} mandated by the U.S. Environmental Protection Agency. Estimated residence times indicated for MAAP groundwater were 56 days for water alone, compared with 6 to 18 days where plants were present, depending on species. Residence times were 55 days in incubations with unautoclaved sediment and 16 days with autoclaved sediment. Correlation analysis indicated that a component of TNT removal from water was plant mediated and that residence time decreased with increasing plant mass.

RDX removal from groundwater was highly variable and not significantly accelerated by the presence of plants or sediment. Extrapolation of a linear regression model to 2 μg RDX L^{-1} (zero order kinetics) indicated residence times of 628 days for groundwater alone, 136 or 1,162 days with sterile or native sediment, and 50 to 298 days with plants at the lower density, depending on species. Correlation analysis indicated that RDX removal from water was not directly plant mediated, but increased with decreasing oxygen concentration in water. This suggests the involvement of facultative and/or obligate anaerobic microorganisms or the action of nonaerotolerant plant enzymes.

End-point tissue analyses showed that TNT was absent in sediments and plants, but that 4-amino-2,6-dinitrotoluene (4ADNT) was present in plant tissue. RDX was present in sediments and plant tissue at the end of the incubation, accumulating up to 200 μg g^{-1} dry weight in plants. Principal component analysis of waterborne TNT and TNT-metabolites indicated variation among species and substrates. No indication of TNT degradation via nitro-group removal was found.

Based on the results of this study, species recommended for deployment in phytoremediation demonstration lagoons at MAAP were elodea, sago pondweed, water star-grass, and curlyleaf pondweed.